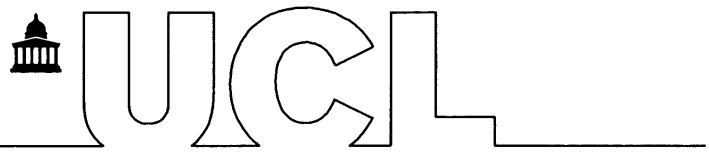

# *New synthetic approaches to prepare degradable polymers*


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**Submitted for a Doctor of Philosophy Degree**

**January 2008**





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# Declaration

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**Name of candidate:**

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**Declaration:**

I Monzur Ali, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed: ..... Date .....

Candidate

Signed: ..... Date .....

Supervisor

# Abstract

This thesis is concerned with the synthesis of acid labile *co*-polymers. Two polymer systems were examined (1) polyacetals and (2) poly(ortho esters). As for poly(ortho esters), there is a need for better synthetic methods to prepare these polymers more easily without the need of stringent anhydrous conditions, with more broad structural variation, and in a more cost effective manner.

Pendent functionalised polyacetals derived from PEG and tyrosine derived monomer diols have been prepared and their structure activity relationships determined. A smaller size alkyl chain on the tyrosine derived monomer diol increased the rate of degradation of these polyacetal libraries.

For poly(ortho esters), a first strategy involved the preparation of novel stable orthoester monomers. The key aspect was to embed the orthoester within the monomer while providing orthogonal polymerisation functionality. This synthetic route attempts to address the synthetic limitations for the preparation of existing poly(ortho esters) and it is believed to be the first such example. The stabile symmetrical bicyclic [2.2.2] orthoester monomer molecule derived from the naturally occurring metabolite phenyl acetic acid was used to prepare the new poly(ortho esters). The bicyclic orthoester [2.2.2] ring arrangement provided the monomer with rigidity, therefore enabling a pure solid monomer to be prepared in three synthetic steps. This approach provided a more efficient polymerisation reaction that requires less stringent polymerisation reaction conditions then existing literature examples for preparing poly(ortho esters).

The second broad strategy examined the synthesis of a hydrolytically stable precursor poly (oxetane esters), which underwent a pH triggered rearrangement reaction within the polymer mainchain to prepare orthoester moieties in the polymer mainchain. In conclusion these strategies have provided new synthetic examples of preparing the highly degradable acid labile polymers e.g. poly(ortho esters).

**This thesis is dedicated to my father.**

# Acknowledgements

First of all, I would like to acknowledge my supervisors professor Steve Brocchini and professor Jonathan Knowles for their support and guidance throughout this research. Thanks to Steve for his patience and original idea development during the research. I also like to acknowledge Dr Malcolm Brown, Smith and Nephew plc and Engineering Physical Science Research Council for their financial support for the research.

I am grateful to colleagues and friends in the biomedical polymer group past and present, especially Dr. Jenny Rickerby, Dr. Manu Porssa, Dr. Sibu Balan, Dr. Debbie Harris and Stefan Salomon for their help and assistance. A special thanks to Dr. Anthony Godwin, Dr Norbert Rumf and Dr Mathew Benton for their synthetic chemistry skills, knowledge and assistance. Many thanks, to Dr. Mira Zolh and the technicians at The School of Pharmacy (University of London), Eastmen Dental Institute (UCL) and Smith and Nephew Plc.

On a personal note I would like to thank my family (Sahid Ali, Karful Nassa, Juned Ali, my wife Raifa Sultana, and my friends (Rey, Sads and rest of the boys).

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# Lists of schemes, figures and tables

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# Abbreviations

| | |
|---|---|
| CSA | camphor sulphonic acid |
| DCC | dicyclohexylcarbodiimide |
| DIEA | N,N-diisopropylethylamine |
| DSC | differential scanning calorimetry |
| DMF | dimethylformide |
| DMAP | dimethyl amino pyridine |
| DMSO | dimethylsulfoxide |
| EI | electron ionisation |
| Eq. | reactive group molar equivalents |
| Fmoc | 9-fluorenylmethoxycarbonyl group |
| FT-IR | fourier transform-indra red |
| Gly | glycine |
| GMP | good manufacturing practice |
| GPC | gel permeation chromatography |
| HOBT | 1-hydroxybenzotriazole |
| IR | infra-red |
| m-CPBA | meta-Chloroperoxybenzoic acid |
| MeOH | methanol |
| Mn | number average molecular weight |
| Mw | molecular weights |
| MWD | molecular weight distribution |
| n-BuLi | N-butyllithium |
| NMP | N-methyl pyrrolidone |

| | |
|---|---|
| NHS | N-hydroxysuccinimide |
| PDI | polydispersity index |
| PEG | poly (ethylene glycol) |
| POE's | poly(ortho esters) |
| PMMA | poly(methyl methacrylate) |
| p-TSA | para-toluene sulphonic acid |
| $R_f$ | retension factor |
| RT | room temperature |
| SEM | scanning electron microscopy |
| TFA | trifluoroacetic acid |
| Tg | glass transition temperatures |
| THF | tetrahydrofuran |
| TMEDA | tetramethylethylenediamine |
| TLC | tin layer chromatography |
| Z- | benzyloxycarbonyl group |

# Chapter 1

## *General introduction*

# Chapter 1

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## *General introduction*

### *1.0 Introduction: Acid labile biodegradable polymers and the need for improved synthetic methodologies*

Polymers are widely utilised in healthcare products including final dosage forms of most orally administered medicines to more complex biopharmaceutical formulations, drug delivery systems and biomaterials for load bearing and tissue engineering applications[1]. The synthesis and properties of polymers differ significantly from smaller, low molecular weight molecules. In comparison to small organic molecules, polymers are prepared by reactions where many bond forming reactions must occur for the polymer to be formed. Furthermore, the small monomer molecules are required to be extremely pure for a successful polymerisation, and achieving high molecular weight polymers.

The challenge undertaken in this thesis is to develop synthetic methodologies that can be used to prepare hydrolytically degradable, acid labile biomedical polymers that can be designed to address specific biomedical applications, e.g. orthopaedic or drug delivery applications etc. Many existing biomaterials, particularly acid labile polymers are unable to meet the specifications for their applications. Often their design and synthesis are not based around the specific clinical needs or the end application specifications. Examples include the use of polyester, poly (glycolic lactic acid) used in orthopaedic application, where the polymeric material degrade in a bulk erosion processes, and not hold its mechanical strength throughout the degradation process of the load bearing application[2]. Frequently, optimising polymer properties is limited because of synthetic limitations. Polymer property limitations are often further exacerbated for degradable polymers. While several classes of polyesters have been prepared, the synthesis of more labile polymer systems based on acid labile moieties such as acetals or orthoesters, are more

limited. The design, synthesis, and commercial development of new acid labile biodegradable polymers needs to be based on the biological rationale, and specific clinical specifications.

### *1.1 Biological rationale for the synthesis of acid labile co-polymers*

Acid labile biodegradable polymers that undergo degradation characterised by surface erosion processes are finding more clinical applications. Examples of surface eroding acid labile polymers include the poly(ortho esters) Biochronomer® used in a number of drug delivery applications e.g. post-surgical pain relief, anti-inflammatory post orthopaedic surgery to anti-inflammatory in osteoarthritis [62]. The stomach is a key acidic site in the body. Also, diseased tissues tend to be a slightly acidic pH rather than basic pH. Often sites of inflammation including infections and malignancy are acidic. Intracellular sites within the endocytic pathway are also acidic[3]. There are more acidic environments within the human body than basic environments. These are a few examples why acid labile polymer research attracts interests. It is important that the degradation products released from the degradation of the acid labile polymers are biocompatible. Polymers that do not degrade by an autocatalytic processes are desirable, as the degradation to some degree can be controllable. The biocompatibility of the polymer is an important factor for a medicine that is administered or a device that is implanted where the polymeric components can enter the systemic circulation[1]. In such cases, the tissue and cellular uptake can result in polymer accumulation. Polymer degradation products should be designed to be small water-soluble molecules can be washed out of the body, preventing polymer accumulation within tissues and the human body generally[4;5].

## *1.2 Synthesis of acid labile co-polymers using the traditional synthetic method*

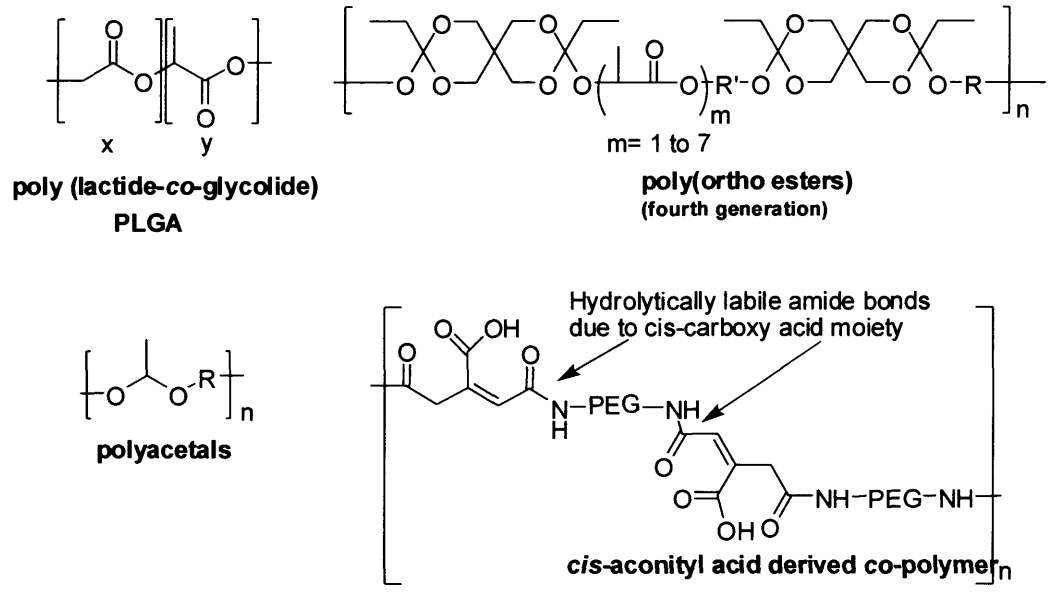



**Figure 1:** Existing examples of the acid labile *co*-polymers including polyacetals, PLGA (polyesters), poly(ortho esters) and cis-aconityl acid *co*-polymers [6-10].

Existing biomedical polymers that can undergo degradation to varying degrees at enhanced rates at acid pH values include: polyacetals, polyesters, *cis*-aconityl derived *co*-polymers, and poly(ortho esters) (Figure 1). Polyesters especially the poly (glycolic-lactic acid (PLGA)) are routinely synthesised by the polycondensation of diacids and diols, self-polycondensation of hydroxyacids, or by ring-opening polymerisation of cyclic diesters, lactones, glycolids and lactides [6-10]. The ring-opening polymerisation (ROP) method can be used to prepare high molecular weight polyesters. In some cases these polyesters can have a narrow molecular weight distribution (MWD). For example, molecular weights high as 42, 000 g $mol^{-1}$ (Mw/Mn = 1.05) have been prepared using thiourea catalysts[11]. Polyesters that are in current clinical use as a carrier vehicle for drug delivery application include: poly (lactide-co-glycolide) (PLGA), under the trade name of Zoladex®. This medicine is injected subcutaneously into the abdomen to deliver as a depot the luteinizing hormone analogue, goserelin acetate, for the treatment of prostate cancer[12]. Although PLGA is clinically used (e.g. sutures[13]) and it has been intensively studied, it is also true that these polymers do not possess optimal properties for all possible applications. For example, devices fabricated from PLGA tend to undergo

degradation by bulk processes which can lead to drug dumping. They are also susceptible to acid driven autocatalysis resulting in non-linear degradation and the release of acidic degradation products that can cause local inflammation[14;15]. Non-linear degradation rates also cause polymer properties to change at non-linear rates (e.g. mechanical properties). Often polyesters are only soluble in organic solvents. They possess a low Tg which can limit some pharmaceutically relevant processing techniques (e.g. spray drying). Polyesters such as the copolymer 3-hydroxybutyrate-*co*-3-hydroxyvalerate is marketed as Biopol®. This product has been used as sutures and drug delivery applications, because this material has better processing characteristics than PGA[13;16].

There continues to be a need for new rationally designed acid labile *co*-polymers to add to the current biomedical polymer library that are reported within the literature[5;17]. Such polymeric materials would provide an opportunity to better match the polymer to specific medical applications. Minimum specifications require that new materials be biocompatible, broadly degradable by erosion and hold their mechanical integrity for load bearing applications throughout the degradation processes.

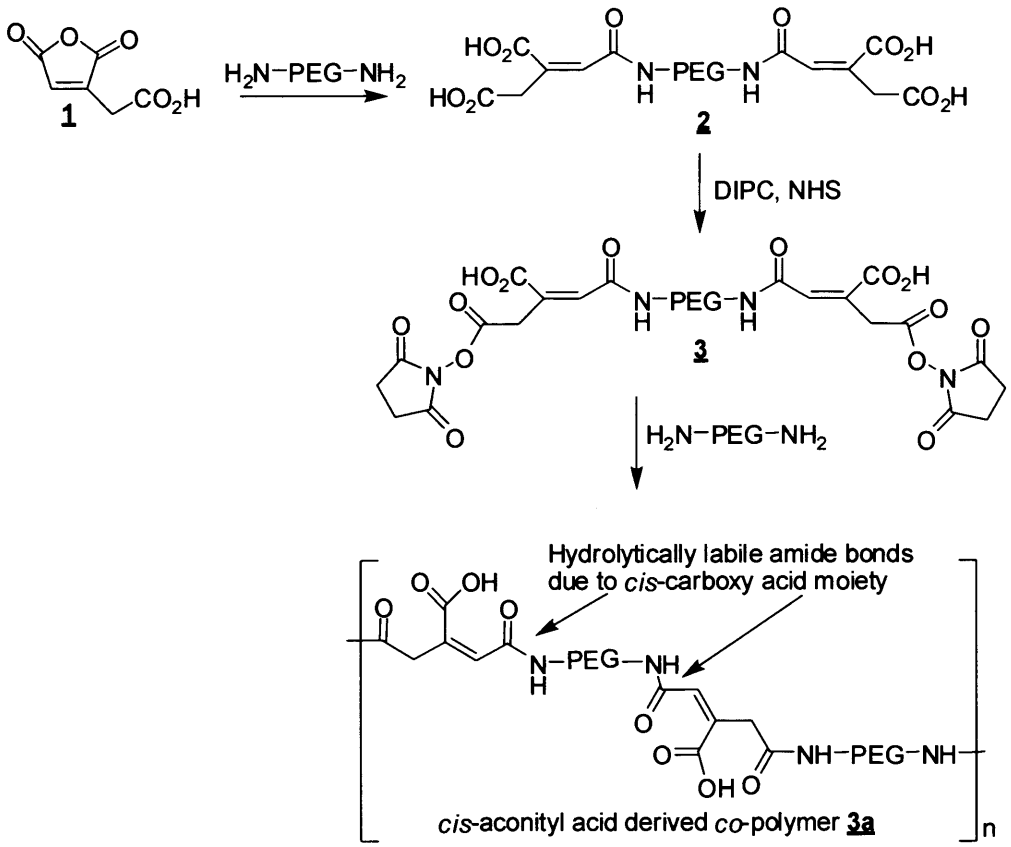
Polyesters prepared from chiral L-lactic acid PLLA have high mechanical properties such as tensile strength and modulus. Thus it has found widespread use in load bearing orthopaedic applications[18]. PLLA based orthopaedic products that are available on the market include [19]:

1. Phantom soft thread soft tissue fixation Screw®
2. Phantom Suture Anchor®
3. Full Thread Bio Interference Screw®
4. BioScrew®
5. Bio-Anchor®
6. Dexon® and Dacron® [20;21]
7. Sculptra®

Polyesters such as PLLA unfortunately do not posses optimal properties for a number of medical applications. These polymers have a tendency to cause

inflammatory responses, due to their autocatalytic bulk degradation processes. PLLA being more hydrophobic then PGA, it therefore degrades at a slower rate and this is particularly true for the very high molecular weight PLLA. This can take between 2 and 5.6 years for total resorption in *in vivo* studies[18;22]. The rate of degradation is dependent on the degree of polymer crystallinity as well as the porosity of the polymer matrix. Even though the polymer loses almost all its mechanical integrity within approximately 6 months, during which it is hydrolysed, there are no further significant changes in mass loss occurring over a much more extended period of time[19]. Therefore, current interests among these polymers include the preparation of a mixture of both L-lactic and L-glycolic acid as bioresorbable implant even though this material was first discovered in the 1970's[23]. The fact that these polyesters whether its PLLA, PGA or PLGA degrade by bulk processes limits their diverse clinical applications. The underlying problem of bulk erosion of polyesters still remains a significant obstacle that needs to be addressed. This holds true for both current research and existing research in preparing polyesters. This further emphasises that there is a clear need for new acid labile polymers and *co*-polymers, that address and better match the clinical need, which existing acid labile polymers and specifically polyesters do not satisfy. Furthermore, degradable polyesters such as PLGA do undergo acid catalysed degradation reactions, but do not undergo rapid degradation reactions in acidic conditions. However, there are examples of acid labile polymers reported that are designed to degrade at enhanced rates in acidic pH values. All these polymers are prepared in such a way that their degradable element is prepared during the polymerisation reaction. Which in its self has their own limitation such as polymerisation efficiency e.g. polymer degradation.

Clochard et al have shown an example of cis-aconityl acid derived *co*-polymers prepared with the degradable element in the main chain formed during the polymerisation [24]. The initial step in the polymer synthesis involved a ring-opening reaction of cis-aconitic anhydride **1** with diamines and diamino to produce the precursor monomer **2** (Scheme 1). The free carboxylic acids of precursor monomer **2** was activated using carbodiimide coupling reaction with an bis-(N-hydroxy) succinimide to form the activated disaster macromonomer **3** (Scheme 1). Self-emolative degradation results by neighbouring group assistence of the cis-aconityl carboxylic acid to catalyst amide hydrolysis.




**Scheme 1:** Synthesis of cis-aconityl acid derived *co*-polymers **3a**.

### *1.3 Polycondensation reactions*

Degradable polymers are routinely prepared using the delicate polycondensation method e.g. carbodiimide assists coupling reactions [25]. This is due to the sensitivity of the degradable element (acetal, orthoester or cis-aconityl) formed during the polymerisation reaction degrading. The types of polymerisation that involve harsh reactions conditions are avoided due to potential polymer degradation e.g. high temperature melt polymerisation. This particularly holds true for sensitive monomers that are used in these types of polycondensation. Furthermore, examples of preparing acid labile *co*-polymers that deviate from current method that involve the degradable element formed within the polymerisation have not been reported. This is mainly due to the fact that preparing these types of *co*-polymers is synthetically challenging, and often resulting in limited progress. However, current efforts in degradable polymer synthesis have been focused on designing and synthesizing polymers with tailor

made properties for specific clinical applications by: [19;26]

1. Designing and preparing novel synthetic polymers with unique chemistries to increase the diversity of the polymer structure.

2. Developing biosynthetic processes to form biomimetic polymer structures.

3. Adopting combinatorial and computational approaches in biomaterial design to facilitate the discovery of novel resorbable polymers[27].

Generally, polycondensation is a difficult task compared to chain growth polymerisations, since very high molecular weights can only be achieved at very high conversion rates (> 98 – 99 %) (Figure 2)[25]. A precise stiochiometric balance of the reactive species with the monomers are required, for a successful and efficient polycondensation reactions. This therefore, can be resolved using pure A-B type monomers. However, if a new AA monomer is developed, it would be most efficient to polymerise it with existing BB monomers that can be easily obtained in high purity.

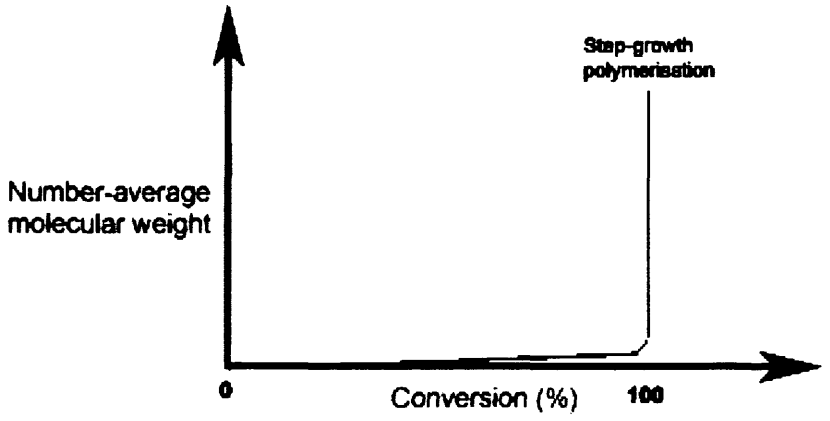



**Figure 2:** Number average molecular weight of step-growth polymerisation, showing high molecular weight achievement at high conversion rate.

### *1.3 Acid labile polymer degradation and erosion*

Polymer degradation is a process defined as a chemical reaction that results in the cleavage of main-chain bonds producing short oligomers, monomers and other low molecular weight degradation products [28;29]. A polymer is considered biodegradable if the degradation process is due to environmental actions, either by bio-catalytic processes (involving bacteria, fungi, enzymes etc) or chemical and radical processes alone (hydrolysis, oxidation, and UV radiations etc)[30;31]. Many factors are known to influence the degradation rate of a polymer. Chemical structure, polymer morphology, molecular weight, and surrounding environment (e.g. pH and temperatures) can all have a significant effect on the degradation rate[2;29;32].

Hydrolytic degradation is caused by the reaction of water with labile bonds (e.g. esters, orthoesters etc.) in the polymer main chain. Hydrophilic polymers take up more water and therefore degrade faster compared to hydrophobic matrices[29]. Erosion of a polymer is defined as the physical disintegration of a polymer as a result of degradation. There are essentially two degradation mechanisms and the polymer degradation can be classified as either bulk or surface eroding. If the water penetration occurred slowly compared to the hydrolysis processes, then mass loss is confined to the surface of the device only[33]. This behaviour is found only in surface eroding polymers. In the case of bulk eroding polymers, water imbibation occurs relatively more quickly than mainchain hydrolysis. For many biomedical applications, surface eroding polymers in principle are more desirable due to the predictability of the erosion of the device, thus providing more control over the degradation processes.

Among the acid labile biodegradable polymers, poly(ortho esters) have generated much interest due to these polymers being able to undergo degradation processes characterised by surface erosion processes (Figure 3)[34;35]. Surface eroding poly(ortho esters) can potentially maintain their properties throughout the degradation process. Therefore, poly(ortho esters) have been actively researched, specifically in the field of drug delivery[36;37]. The controlled surface eroding properties of poly(ortho esters) have been extensively utilised to prepare pharmaceutical formulations that are designed for controlled release, which is

governed by the polymeric rate of degradation[38;39].

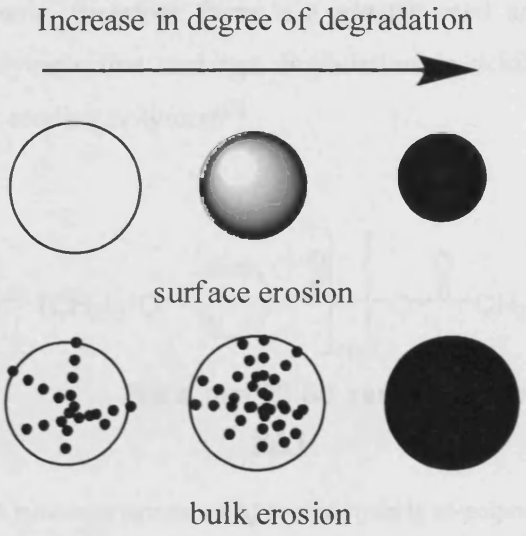
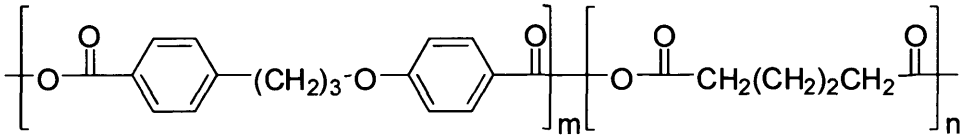



**Figure 3:** Comparison of surface erosion against bulk erosion, where degradation increases from left (white circle) to right of the diagram (black circle).

An example of a surface eroding polymeric material that is primarily used for the purposes of drug delivery is the polyanhydrides based product Gliadel wafer® [40]. It should be noted that the polyanhydrides are the only example of a non acid labile polymer, that is widely accepted to undergo degradation characterised by surface erosion processes [41]. Langer et al were the first research group that utilised this advantageous surface eroding properties for drug delivery applications [42]. The anhydride bonds react rapidly with water to produce two acidic fragments. By varying the individual monomers used to prepare polyanhydrides, their properties and degradability can be modified, similar to existing poly(ortho esters) (e.g. poly(ortho esters) **4** ) [43]. One such *co*-polymer combination, P (CPP:SA, 20:80 ratio) **PA1**, has been used as an excipient for the sustained release of chemotherapeutic agent, Carmustine (Figure 4). This product is available on the market under the trade name of Gliadel wafer® and it is used in the treatment of brain tumours. This product was developed and marketed by MGI Pharma [40]. Polyanhydrides particularly, Gliadel wafer® has a limited therapeutic application range, because the polymer undergoes erosion in a basic environment[44]. Although research interests have been generated through the product Gliadel wafer® available on the market, these polymers are studied less primarily due to the polymer

degradation occurring in basic pH environment [40;44;45]. Furthermore, the polyanhydrides are difficult to synthesise. The human body contains more acidic environments than basic, therefore there is a greater need and larger number of application(s) for polymers that undergo degradation in acidic pH environments, especially for surface eroding polymers[3].




Ratio m:n 20:80; random polymer

**PA1**

**Figure 4:** Polifeprosan 20 molecular structure **PA1** (polyanhydride co-polymer) [40].

Generally, acid labile polymers and *co*-polymers that are prepared using existing examples of synthetic methods that have been reported. The synthetic method involves the acid labile degradable element formed during the step-wise polymerisation (polycondensation reaction) to form the polymer or the *co*-polymer respectively (Figure 5). This FIGURE shows a stepwise polymerisation used to prepare an example of a poly(ortho esters). The synthesis incorporates the highly degradable element of the polymer (e.g. the orthoester bonds) to be formed in the polymerisation step. Current examples of poly (ortho esters) synthesis involves the design, the synthesis and the final polymer properties to be controlled by this stepwise polymerisations, as shown in FIGURE 5. The examples of final polymer properties include: the molecular weight, mechanical, thermal and processable properties.

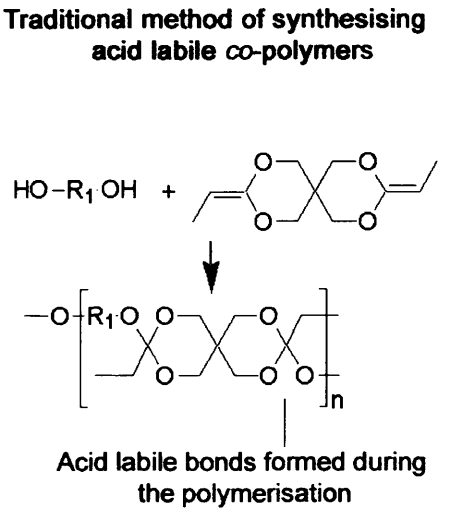



**Figure 5:** Existing synthetic example of preparing acid labile poly (ortho esters), with the degradable element of the polymer formed during the polymerisation.

### *1.4 Poly(ortho esters)*

There are four generations of poly(ortho esters) that have been prepared over a period of 30 years (Figure 6)[46]. Poly(ortho esters) have been studied with the aim to produce a biomaterial for clinical application, but this has been met with limited success.

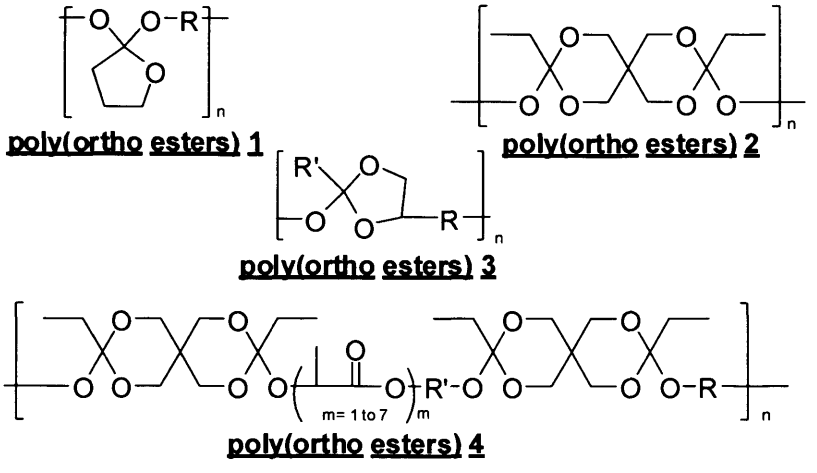



**Figure 6:** Polymeric structure of the four existing generation of poly(ortho esters) 1 – 4 [46].

The development of existing classes of poly(ortho esters) was first investigated by Alza® Corporation and then SRI International® [47-51]. In designing these polymers, the desirable properties required were based on the application specification(s):

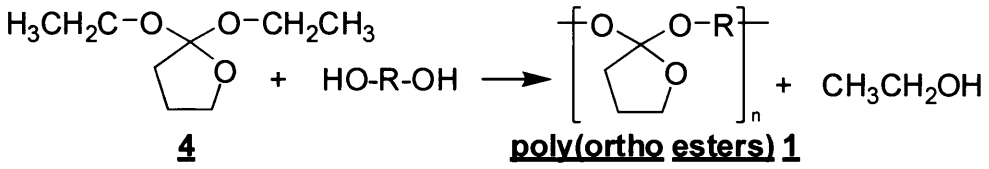
- Polymer should degrade hydrolytically, to small water soluble products that are toxicologically benign and can be eliminated from the host.
- The rate of hydrolysis can be controlled over a long period, by simple manipulation of the structure or the use of additives(s).
- Polymer should erode via surface erosion processes.
- Adjustable physical properties achieved by structural alteration.

### *1.5 Synthesis of existing poly(ortho esters)*

**Poly(ortho esters) 1**

**Poly(ortho esters) 1** was synthesised by an acid catalysed transesterification reaction between a diol and diethoxytetrahydrofuran **4** (Scheme 2)[46-51]. Initial development work showed monomers with three alkoxy groups would inevitably result in a cross-linked polymer. Therefore, one alkoxy group was suppressed by using a cyclic monomer, in order to achieve linear poly(ortho esters)[52]. This first generation of poly(ortho esters) was abandoned due to the difficult issues associated with the polymer synthesis and poor polymer physical properties. For example, the polymer had a low glass transition temperature due to the inefficiency of the polymerisation (e.g. spontaneous de-polymerisation)[35]. Also this class of poly(ortho esters) had oil like material properties. The hydrolysis of the polymer produced γ-butyrolactone, which, readily opened to γ-hydroxybutyric acid leading to autocatalytic hydrolysis of the polymer. The autocatalytic hydrolysis were stabilised with basic additives e.g. sodium carbonate, but with very little improvement, which led to limited research and potential biomedical applications development.

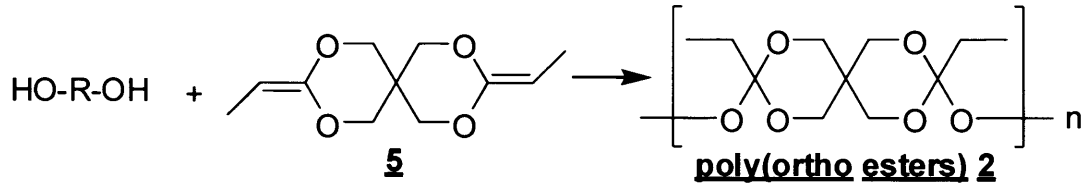
Furthermore, the polymerisation yields ethanol as a *co*-product, which has a great limitation of the synthesis. To get rid of ethanol completely was difficult in the reaction, and small amount of remaining ethanol interfered in pushing the reaction equilibrium forward to 100 %.




**4** **poly(ortho esters) 1**

**Scheme 2:** Synthesis of existing **poly(ortho esters) 1** and the production of ethanol as a *co*-product.

The second generation **poly(ortho esters) 2** was an improvement of the first generation of **poly(ortho esters) 1**, due to a better synthetic method. The synthetic method involved the polymerisation of a diol to a diketene acetal (3,9,-diethylidene-2,4,8,10-tetraoxaspiro[5,5] undecane) **5** , as shown in Scheme 3[46].



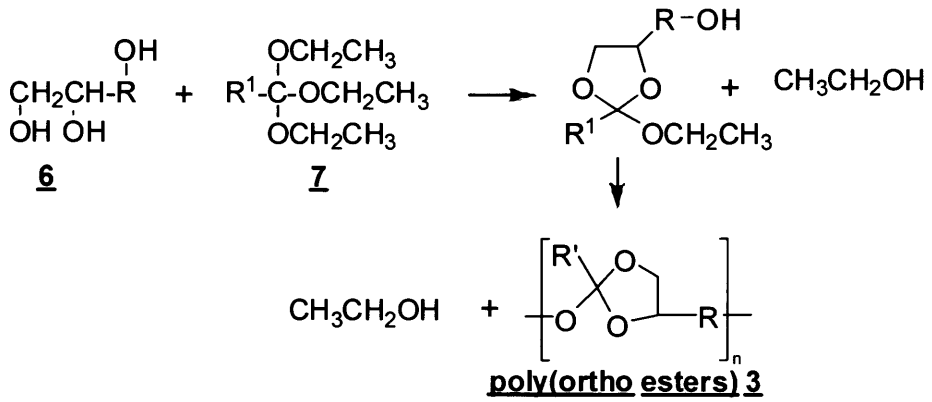

**5** **poly(ortho esters) 2**

**Scheme 3:** Synthesis of existing **poly(ortho esters) 2** prepared by a polymerisation of a diol and diketene acetal (3,9,-diethylidene-2,4,8,10-tetraoxaspiro[5,5] undecane) **5**.

The polymerisation proceeded readily with good reproducibility, and the molecular weight can be adjusted by stiochiometric alteration of the monomer ratios. The hydrolysis of the **poly(ortho esters) 2** were not autocatalytic, because the **poly(ortho esters) 2** hydrolysis produced neutral degradation products[53]. Although this generation of Poly(ortho esters) had generated much research and development interest, the research was not taken further for commercial development[54;55], because, the polymer was too hydrophobic and did not degrade in an acceptable time frame for controlled drug delivery applications[37].

The synthesis of **poly(ortho esters) 3** (Scheme 4) involves an acid catalysed step-wise polymerisation[46;55]. The reaction involved dissolving the triol monomer **6** in a

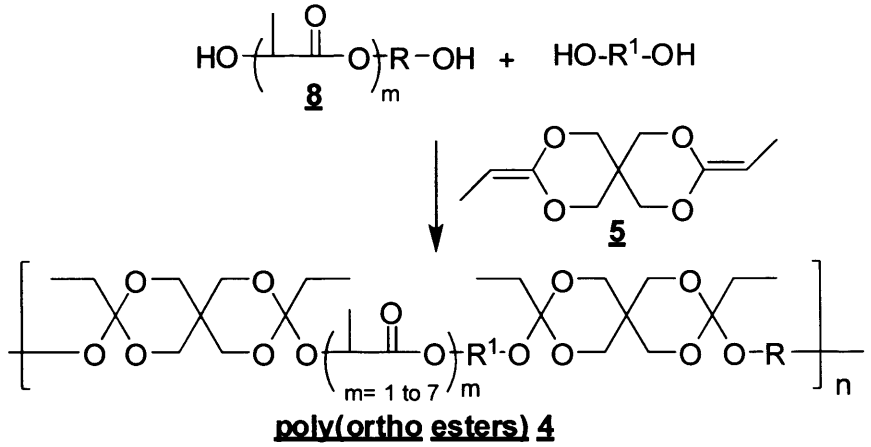
polar solvent (THF) with a commercially available triethyl orthoformate 7 with azo-tropical removal of the by-product ethanol. Unlike **poly(ortho esters) 2** (Scheme 3) where, the polymerisation is instantaneous, the polymerisation of **poly(ortho esters) 3** takes a significant amount of time and reproducibility is difficult to achieve. Achieving high molecular weight is extremely difficult as complete removal of ethanol (by-product) is required. Therefore, depolymerisation occurs readily. Also, the material had insufficient mechanical and thermal properties, because of the flexible nature of polymer backbone producing a semi-solid material at room temperature. This limited its broad medical applications, and was not further developed[56]. Because, of the following difficult issues: (1) difficulties in the synthesis, (2) difficulties in reproducibility and (3) oil to gel like physical properties.




**Scheme 4**: Synthesis of existing **poly(ortho esters) 3** prepared by a polymerisation of a triol monomer **6** and the commercially available triethyl orthoformate 7.

The synthesis of **poly(ortho esters) 4** (Scheme 5) is similar to the synthesis of **poly(ortho esters) 2** (Scheme 3). **Poly(ortho esters) 4** was the most improved version in term of the synthesis of the four families of Poly(ortho esters)[35]. The synthesis involved an acid catalysed step-wise polymerisation between a diol, a diketene acetal (3,9,-diethylidene-2,4,8,10-tetraoxaspiro[5,5] undecane) **5** and a short segment of a latent acid diol **8** of either glycolic acid or lactic acid (Scheme 5) [57;58]. The polymerisation was carried out in polar solvents and the reaction proceeds almost instantaneously. This polymer had desirable properties such as: controlled erosion rates, and mechanical and thermal properties that can be varied by using a variable latent acid diol (e.g. glycolic acid or lactic acid diol). This reaction is highly reproducible and kilogram quantities of GMP polymer can be produced. The

polymeric device is market by AP Pharma under the trade name of Biochronomer® [34;36;59]. The physical form of the polymer material can be prepared in a variety of forms, ranging from hard, glassy materials to materials that are injectable at room temperature by proper selection of diols. Biochronomer® bioerodible materials as a drug delivery system has advantages over conventional drug delivery fabrication (i.e. where polymer is used) as drugs can potentially be incorporated with ease. The drug incorporation is achieved by simple room temperature mixing of the drug with the polymeric material, and the formulation commercialized in pre-filled, sterile syringes[35]. **Poly(ortho esters) 4** based drug delivery device Biochronomer® is currently in clinical trials (Phase II in September 2005), submitted by AP Pharma. The projected drug delivery applications of this particular polymer are in the treatment of post-surgical pain and ophthalmic diseases[26;34].




**Scheme 5**: Synthesis of existing **poly(ortho esters) 4** prepared by polymerisation of a diol, a diketene acetal (3,9,-diethylidene-2,4,8,10-tetraoxaspiro[5,5] undecane) **5** and a short segment of a latent acid diol **8.**

AP Pharma have developed a number of products that are currently undergoing clinical trials at various developmental stages using the **poly(ortho esters) 4** based drug delivery systems (Table 1)[59]. As a result of these products being developed by AP Pharma, there are a number of research groups actively undertaking research based around developing POE based drug delivery system. It can be seen from the range of products developed by AP Pharma that poly(ortho esters) are a set of useful polymers that can potentially undergo chemical modification that allow the polymer to undergo degradation at different rate(s). The flexibility of altering the degradation

rate has led to a number of potential product applications, from drug release of days to just over months (Table 1). Furthermore, there has been evidence within the literature that the degradation rate and rate controlled drug release from the system can be stretched to well over a year[53].

*Table 1*: Current poly(ortho esters) based products in clinical development by AP Pharma plc [59].

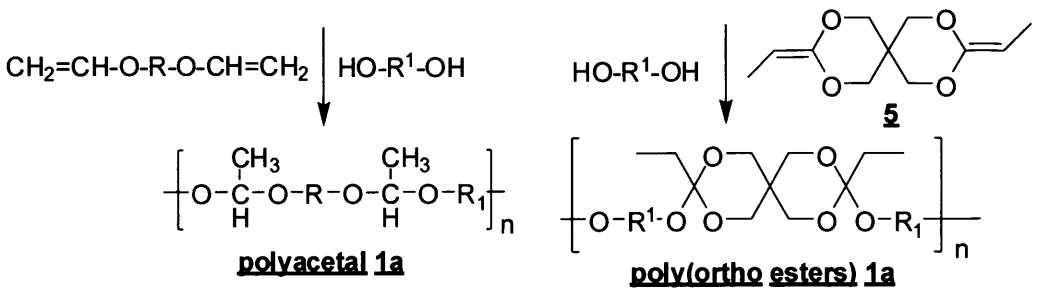
| Product Candidate | Potential Application | Drug | TargetDuration | Status |
|---|---|---|---|---|
| **APF112** | Post-surgical pain relief | Mepivacaine | Up to 36 hrs | Phase II |
| **APF580** | Pain relief | Opiate | Minimum 7 days | Preclinical |
| **APF328** | Local anti-inflaminatory (orthopaedic surgery) | Meloxicam | Up to 2 weeks | Preclinical |
| **APF505** | anti-inflaminatory (orthoarthritis) | Meloxicam | Up to 6 weeks | Preclinical |

However, **poly(ortho esters) 4** have synthetic problems that still needs addressing. For example, the synthetic method requires extremely anhydrous reaction conditions (e.g. dry-box) And this hinders more efficient commercialization of the polymer, as expensive specialized equipment and techniques are required to scale-up production. Furthermore, current poly(ortho esters) (e.g. **poly(ortho esters) 4** ) that have been developed and prepared have low mechanical properties and in particular, these materials have a tendency to be an oily or viscous material. This includes the **poly(ortho esters) 4** based products developed by AP Pharma (Biochronomer®)[59]. Because of the unique material properties that Biochronomer® has it is limited to a narrow range of product development and applications. Therefore, the addressing of this limitation has been actively looked at within the literature, to problem solve and find an alternative better matching poly(ortho esters) material(s)[34;37]. With the aim of this these materials potentially be used in an number of biomedical applications. Where the polymeric materials are specially designed for fit for purpose, with high strength, modules, and more efficient polymer fabrication properties then existing poly(ortho esters).

## *1.4 Polyacetals*

In protective group chemistry it is well-known that acetal(s) are formed by undertaking a reaction of an aldehyde and an alcohol[60]. This behaviour was used to prepare polyacetals using an aldehyde and a diol to prepare polyacetals with side chains. Polyacetals are routinely prepared through the traditional method of polycondensation techniques (Figure 5). This traditional polymerisation technique involves forming the degradation element of the polymer simultaneously in the polymerisation steps, while the polymer is growing.

Polyacetals have generated much interest in particular in the field of drug delivery as an acid labile polymer[61-63]. There have been a few reports of preparing highly acid labile polymers with high molecular weight using a *ter*-polymerisation, which is used as a carrier to delivery chemotherapeutic agents[3;64;65]. Furthermore, these polyacetals generate small water soluble degradation products, which are easily excreted out of the human body, thus making these polymers to be bioresorbable. Heller et al have studied a series of graft *co*-polymers where poly(ortho esters) and a mixture of polyester (PLGA) were prepared within the backbone of the polymer[66]. The authors stated these *co*-polymers were not optimal because the polyesters made the *co*-polymer hydrophilic. Therefore, the hydrolysis rates of these *co*-polymers are difficult to control, thus the authors used a polyacetal polymeric linkage within the backbone of the polymer. It was hoped that the hydrophobicity increase within the *co*-polymer would help to control the degradation rates. Furthermore, the polyacetals preparation included similar synthetic chemistries as used in the preparation of **poly(ortho esters) 4** (Scheme 5), as mentioned previously. The chemistry involved includes an acid catalysed reaction, involving a nucleophillic attack of the hydroxyl group of the diol towards the alpha carbon of the vinyl ether (Scheme 6). This prepared *co*-polymers of **polyacetals 1a** and **poly(ortho esters) 1a** (Scheme 6). These types of *co*-polymers lacked high mechanical strength and rigidity that is a requirement for materials that have good processibility properties. The materials were oily to gel like in physical appearance.




**Scheme 6**: **Polyacetals 1a** prepared by polymerisation of a divinyl ether and a diol.
**Poly(ortho esters) 1a** prepared by a polymerisation of a diol and a diketene acetal (3,9,-diethylidene-2,4,8,10-tetraoxaspiro[5,5] undecane) **5**.

To address the limitation of the polyacetals *co*-polymers prepared by Heller et al, the monomers need redesigning[66]. The new sets of monomers need to incorporate rigid characteristics within the design. This would enable when monomers are *co*-polymerised to prepare polymers with high mechanical strength and good processibility.

# *Aims*

From the studies and the considerations that have been described in this introduction, it is clear that there are needs both for (1) new degradable polymers that can undergo degradation at enhanced rates at slightly acidic pH values and (2) new synthetic methodologies to prepare degradable polymers, especially poly(ortho esters).

Polyacetals derived from PEG and tyrosine derived diols will be prepared to determine their structure activity relationships. Specifically it is critical to determine how the properties of polymers with a relatively large component of their structure derived from PEG will vary with defined structural changes in their tyrosine diol monomer.

For poly(ortho esters) there is a need for an efficient synthetic method to address the synthetic limitations of existing poly(ortho esters). To achieve this objective, we hypothesise that it should be possible to incorporate the degradable orthoester element within the monomer. Different functionality in the monomer will be utilised for polymerisation. Optimally, amine functionality will be used for polymerisation. Amine basicity will not cause degradation of the orthoester moiety. Less optimally, since polymerisation chemistry is generally less efficient in mild conditions, we will examine hydroxy functionality for polymerisation.

It is hoped that this approach will allow the preparation of poly(ortho esters) with reaction conditions that can be scaled economically and will allow the practical optimisation of poly(ortho esters) structure-property correlations that could be matched to specific applications. A second synthetic strategy will also be examined to prepare POEs. This second strategy is based on the concept of conducting a reaction on a preformed polymer to give orthoester moieties within the polymer mainchain. The aim being to prepare a stable precursor polymer that could be made to be more hydrolytically degradable.

# Chapter 2

## *Design & synthesis of polyacetals*

# Chapter 2

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# *Design & synthesis of polyacetals*

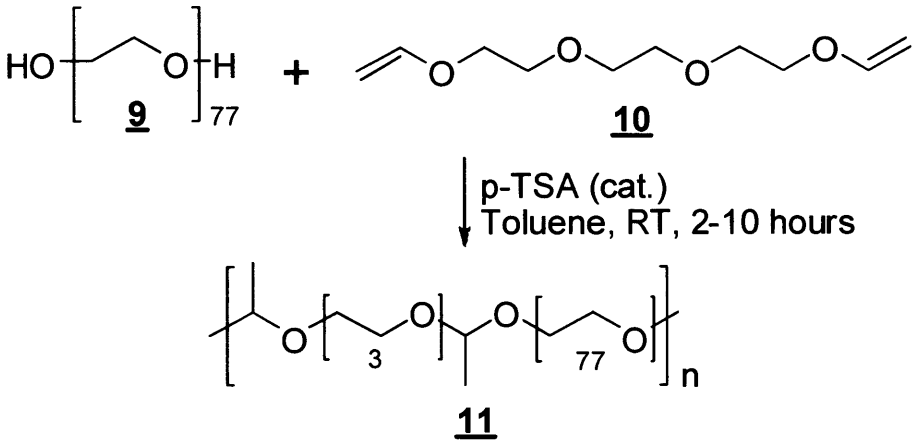
## *2.1 Introduction: Traditional synthetic method used to prepare polyacetals*

Heller et al have described the preparation of polyacetals by an acid catalysed polycondensation reaction of divinyl ether with either dihydroxy aliphatic or diphenolic monomers[67;68]. Others have prepared water-soluble polyacetals with the incorporation of diphenolic diethylstibesterol (DES) in the polymer main chain[69]. However, DES is rarely used in the clinic due to the toxic nature of the diphenols. A study by Vicent et al showed DES derived polyacetals had a greater cytotoxicity than DES against human and murine tumour cell lines ( IC50 =48 and 420 mug/ml against MCF-7 human breast cancer cells and IC50 =97 and 560 mug/ml against B16F10 murine melanoma cells, respectively). Due to the toxic nature of DES its use in biomedical application is avoided[69]. Examples to prepare divinylether derived polyacetals with diphenolic monomers particularly DES, suggested other diphenolic monomers can potentially be used. We seek to prepare pendent functionalised polyacetals that are derived from diphenolic monomers, to potentially possess a high degree of mechanical and processibility properties.

## *2.2 Results and Discussions*

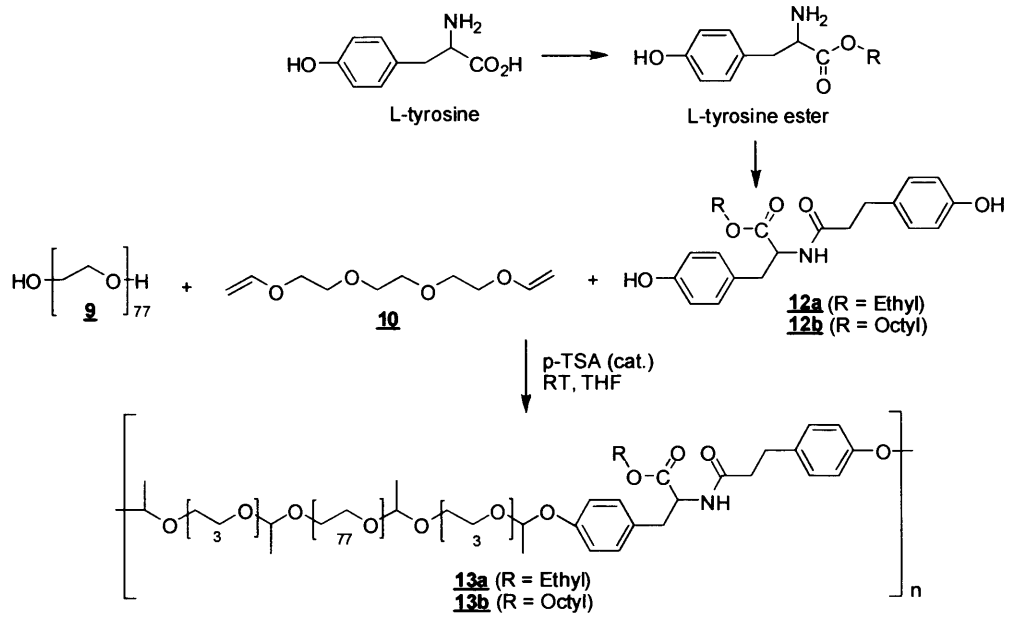
### *2.2.1 L – tyrosine derived polyacetals design and synthesis*

Water soluble polyacetals **11** have been prepared by a *co*-polymerisation using a polyethylene glycol (PEG) **9** diol and a divinyl ether **10** in our research group (Scheme 7)[65;70].




**Scheme 7**: Synthesis of water soluble polyacetals **11** by acid catalysed polymerisation of PEG **9** and triethylene glycol divinyl ether **10**.

Polyacetals **11** (Scheme 7) were prepared using the traditional synthetic method of preparing degradable polymers, where the degradable element is formed in the polymerisation step. The polyacetals **11** had insufficient material properties for a wide range of biomedical applications. These mechanical material properties include: no *T*g values and lower processibility. To address the mechanical property limitation of the polyacetal **11** (Scheme 7), a rigid L-tyrosine derived diphenolic monomer **12a** & **12b** was incorporated into the synthetic design (Scheme 8). We hoped this would increase material processibility and mechanical properties. It should be noted that Kohn *et al* have used L-tyrosine derived diphenolic monomer and PEG **1** as a *co*-monomer to prepare other biomedical polymers[71;72].




**Scheme 8**: Preparation of water soluble polyacetals **13a** and **13b** prepared by a ter-polymerisation of PEG **9**, divinyl ether **10** and a tyrosine derived monomer diol **12a** or **12b**.

The monomers **12a** & **12b** (Scheme 8) were prepared by a carbodiimide mediated coupling reaction of a L-tyrosine ester and hydroxyphenyl-propanoic acid (commonly known as desaminotyrosine)[71;73;74]. *ter*-polymerisation described herein were conducted using PEG **9** (3400 g $mol^{-1}$), the divinyl ether **10** derived from triethylene glycol and the appropriate diphenolic monomer **12a** or **12b** (Scheme 8). Molecular weight characteristics and diphenol incorporation are listed in Table 2. Although the polyacetals **13a** and **13b** were water soluble, the GPC data were obtained in DMF at 70 °C because of a better resolution.

The presence of the degradable acetal bonds found in the polymeric backbone of polyacetals **13a** and **13b** were identified using $^1$H-NMR. The acetal bonds generated from the aliphatic PEG hydroxy groups were assigned to a doublet at 1.2 – 1.3 ppm and a multiplet at 4.4 – 4.7 ppm in the $^1$H-NMR spectrum. The acetals signals were similar to PEG derived polyacetals that were prepared by aliphatic alcohols found in literature[70]. As expected the phenolic derived acetal signals appeared further downfield at 1.4 – 1.5 and 5.4 – 5.5 ppm respectively. The difference in the signal ratio between these two different types of acetal groups produced an estimate of L-tyrosine diphenolic monomer (**12a** & **12b**) incorporation within the polymer **13a** & **13b**.

*Table 2*: List of molecular weight characteristics of *ter*-polyacetals **13a** & **13b** and the levels of diphenols (**12a** & **12b**) incorporation as determined by $^1$H-NMR.

| *Polymer Number* | *Diphenol Monomer* | *Polymer Yield (%)* | *$M_w$ (g mol$^{-1}$)* | *PDI* | *Ratio (4:1)*[1] | *Measured ratio*[2] | *% diphenols 12a (12b) incorporation*[3] |
|---|---|---|---|---|---|---|---|
| P1 | 12a | 97 | 46 000 | 2.0 | 0.75:1 | 0.55:1 | 73 |
| P2 | 12a | 48 | 43 000 | 2.9 | 10:1 | 1.1:1 | 11 |
| P3 | 12b | 93 | 71 000 | 1.7 | 1:1 | 0.71:1 | 71 |
| P4 | 12b | 68 | 24 000 | 1.6 | 10:1 | 5:1 | 50 |

[1]Relative stoichiometry of monomer 12a (12b) and PEG 1. [2]The ratio of the phenolic to the aliphatic-derived acetals as determined by $^1$H-NMR. [3]% of theoretical amount of incorporation.

The effect of stoichiometry of the **12a** (**12b**) and **9** (Scheme 8) on the polymerisation, proved significant in terms of MW and diphenolic monomer incorporation. Higher diphenolic monomer incorporation was evident, when the stoichiometric ratio were lower than to that of PEG **9** alone e.g. polyacetal **P1** and **P3** (Table 2). The low isolated yield of **P2** and **P4** may be attributed to the formation of oligomers during the polymerisation, which precipitated the polymer from the reaction solution (Table 2). Another possibility could have been the acid catalysis may have spontaneously degraded the phenolic acetal, more so than the aliphatic acetal from PEG **9** alone, because phenols are known to be a better leaving group. Complete removal of the acid catalyst (p-TSA) by complexing with a base triethylamine during polymerisation and polymer isolation was crucial for retaining high molecular weights. However, it was difficult to completely isolate the small amount of the conjugated acid-base complex, as it may have co-precipitated during isolation. The complex acting as an impurity may have facilitated auto catalysis activities in the presence of adventitious water leading to polymer degradation during storage.

### *2.2.2 Degradation profile of the L-tyrosine derived polyacetals*

The degradation profile of the polyacetals in general degraded to oligomers as seen by an evenly spaced out regular tail on the GPC chromatogram (Figure 7). The

oligomers were identified as PEG (3,400 g $mol^{-1}$) at the retention volume of 16.1 ml and L-tyrosine diphenolic monomer (**12a** or **12b**) at 19.2 ml. The retention volumes of individual oligomers (PEG **9**) correspond to the degradation oligomers products.

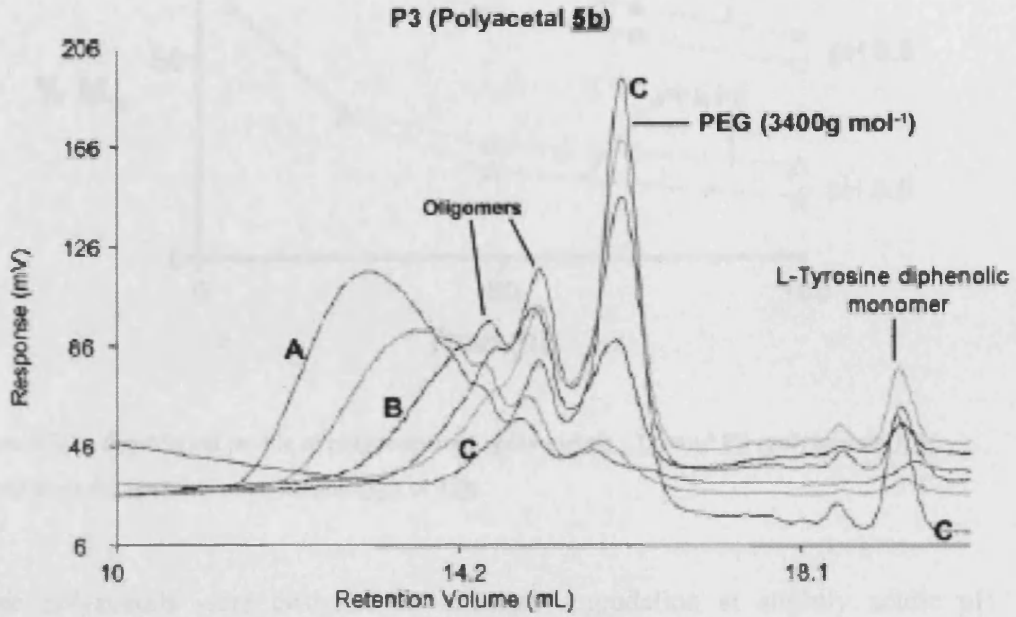



**Figure 7:** GPC chromatogram superposition in DMF for the degradation of **P3 (polyacetal 13b)** at pH 5.5. The time intervals of degradation aliquots collection shown by the labels (A (zero hr), (b (24 hr)) and (C (99 hr)).

The degradation profile of the two polyacetals **13a** and **13b** (Scheme 8) was determined over a degradation period of 4 days at 37 °C in a phosphate buffer at a polymer concentration of 5.0 mg $ml^{-1}$ at the pH values of 7.4, 6.5 and 5.5 (Figure 8). Each polymer samples within the experiment were repeated three times (n=3).

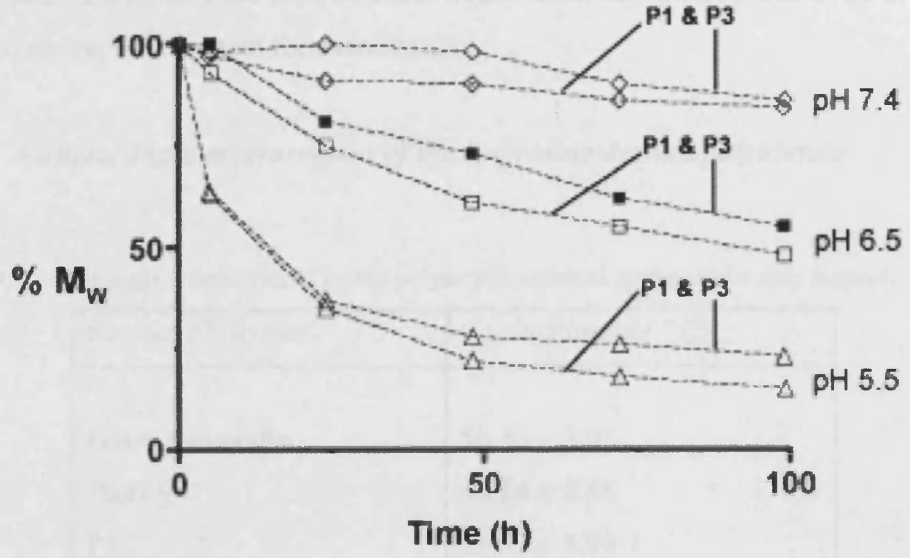



**Figure 8**: The degradation profile of polyacetals **P1** **(polyacetals 13a) and P3 (polyacetals 13b)** derived from the tyrosine monomer diol **12a** or **12b**.

These polyacetals were designed to undergo degradation at slightly acidic pH environments. From the design perspective the polymers under went degradation as expected. The lower the pH environment (e.g. pH 5.5) the more increased the polymer degradation that occurred for both set of polyacetals (**P1** (**13a**) and **P3** (**13b**)) (Figure 8). The polyacetals (**13a** (Scheme 8)) with ethyl ester as the pendent functionality degraded more at the three different pH gradients, due to the hydrophilic nature of the polymer compared to the hydrophobic polyacetal **13b** (Scheme 8). There was a greater than 50 % molecular weight loss within the first day at pH 5.5. These polymer degradation rates appeared to be twice as fast as that observed for the polyacetals **11** (Scheme 7), generated from PEG (3,400 g $mol^{-1}$) and divinyl ether. This behaviour was assumed to be linked to the L-tyrosine derived diphenolic acetal bonds being more susceptible to hydrolytic degradation, as the phenolic group is a good leaving group.

It is widely accepted, that *in vitro* aqueous degradation studies of biomedical polymers involve determining the mass loss of the water insoluble polymer. In our case these polyacetals were water soluble, therefore degradation monitoring was carried out using GPC with DMF solvents. Periodically aliquots of the aqueous

degradation solutions were removed and freeze dried and then re-dissolved in DMF and run on the GPC system for examination.

### *2.2.3 Contact angle measurement of the L-tyrosine derived polyacetals*

*Table 3*: Contact angle measurements for the polyacetals obtained by the sessile drop method.

| Surface / Polymer | *Contact angle ( ° C)* |
|---|---|
| **Glass Coverslip** | 50.56 ± 3.08 |
| **PEG** 9 | 40.84 ± 2.88 |
| P1 | 25.53 ± 5.99 |
| P2 | 22.78 ± 2.77 |
| P3 | 17.77 ± 1.62 |
| P4 | 16.68 ± 1.44 |

The polyacetals were designed as water soluble biomedical polymers. Therefore, the air-water contact angle measurements were examined for polyacetals **P1** – **P4** to study the solubility of these *co*-polymers (Table 3). The air-water contact angle measurements were studied to find out if the surface hydrophobicities differed from PEG **9** alone. The air-water contact angle measurements values were as expected, however, the polyacetals (**P1** – **P4**) generally appeared to be a considerably lower contact angle measurement[75]. These low contact angle measurements of these polyacetals may indicate the existence of a micro two-phase environment[76]. This may possibly be due to the hydrophilic nature of PEG, and the relatively low incorporation of the hydrophobic L-tyrosine derived diphenolic monomer. The PEG regions within the polyacetals may be disrupted or appear at the surface of the polymer, resulting in increased wettability[77]. Therefore, decreasing the air-water contact angle.

### *2.2.4 Preliminary cell attachment study of polyacetals*

Preliminarily cell attachment studies were carried out on the polyacetals using MG63 type osteoblasts cells. The aim of the study was to get an insight into the potential

*in vitro* toxicity and the potential indication of biocompatibility response of these polymers.

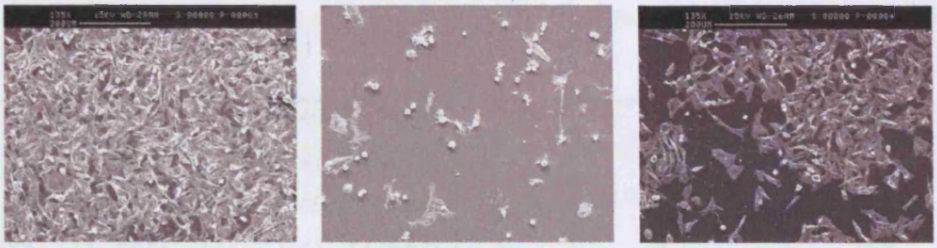



**Figure 9:** Preliminary MG63 Osteoblasts type cell attachment study on L-tyrosine derived polyacetals. Scanning electron microscopy pictures taken at 6 hr time interval. Tissue culture was seeded (1 x $10^4$ cells $cm^{-2}$) onto each cover-slips (SEM pictures).

There was a reduction in the number of cells adherent to the surface of PEG **9** at 6 hr time interval (Figure 9). This was an expected observation. Generally, cell attachment occurred on the surface of all polyacetals (P1 – P4). As observed through scanning electron microscopy (SEM). This indicated that no cell necrosis or heterogeneous distribution of attachment site. At 24 hr all polyacetals surfaces displayed cell characteristics that appeared to be adherent, spread out and proliferating. These *in vitro* study results corroborated prior work that the L-tyrosine derived polyacetals (**P1** – **P4**) were not toxic from a preliminary toxicological evaluation. However, further experiments are required to investigate the full cell viability in contact with these polymers.

### *2.2.5 Mechanical properties of the polyacetals*

The polyacetals **P1** (**13a**) and **P3** (**13b**) mechanical characterisation showed no evidence of glass transition temperatures ($T_g$). However, these two materials showed a crystallisation event ($T_C$) at 33.19 and 25.56 °C (Table 4). Polyacetals **P2** and **P4** on the other hand showed glass transition temperatures but no crystallisation temperatures could be observed. The melting temperatures $T_m$ of all the polyacetals were found to be in the range of 40 – 50 °C. Melting temperatures for **P1** & **P3** are similar but higher then **P2** & **P4**. Heats of fusion are also higher, in particular for **P3** with the highest heat of fusion.

*Table 4*: Glass transition, melting and crystallisation temperatures for the polyacetals **13a** (**P1** / **P2**) and (**13b** (**P3** / **P4**).

| *Polymer* | $T_g$ | $T_m$ | $T_c$ | *Heat of fusion ($J\ g^{-1}$)* | *Crystallinity relative to PEG (%)* |
|---|---|---|---|---|---|
| P1 | - | 49.15 (45.79) | 33.19 | 62.3 | 37.6 |
| P2 | -10.64 (-30.05) | 41.90 (34.15) | - | 41.3 | 24.9 |
| P3 | - | 49.61 (43.64) | 25.56 | 90.2 | 54.4 |
| P4 | -18.08 (-32.12) | 41.33 (35.67) | - | 45.7 | 27.5 |
| PEG $_{3400\ Da}$ | - | 62.42 | - | 165.9 | 100 |

The alternating PEG-tyrosine diphenol polyether reported by Kohn and D'Acunzo, where the L-tyrosine diphenols acted as impurities to lower the crystalline content relative to PEG alone[78]. A decreased melting temperature and heat of fusion was observed for polyacetals **P2** and **P4**, as a result of greater incorporation of the L-tyrosine diphenolic monomer (**12a** or **12b**). For polyacetals (**P1** and **P3**) the increased PEG content may shift the *T*g values to below –50°C (the lower limit of the DSC), therefore, no *T*g was observed for these two polymers.
Although the average molecular number of **P3** is about twice that of **P1**, they both share a similar $T_m$ with **P3** having a higher heats of fusion. This suggests that as molecular weight increases within the polyacetals the influence of PEG increases.

The polyacetals (**P1** – **P4** (Table 2)) were designed and synthesised to possess better mechanical properties than that of the initial polyacetal **3** (Scheme 7). To achieve this aim a rigid L-tyrosine derived diphenolic monomer (**12a** or **12b**) was incorporated into the polyacetals (**13a** or **13b** (Scheme 8)). The L-tyrosine derived polyacetals (**13a** or **13b**) that were prepared could not satisfy this aim completely. This may have been due to the low incorporation of the L-tyrosine diphenolic monomer incorporation into the polymer backbone. Also there is a high degree of PEG content within the polymer backbone. PEG itself is a flexible materials and does not possess a Tg[3].

The preparation of these polyacetals (**13a** & **13b**) (Scheme 8) had difficult issues associated with these polymers.

These difficult issues need addressing before these polyacetals can be taken further, which includes:

1. The low Tg association with the materials possibly due to higher incorporation of the PEG content.
2. Low crystallinity may have been due to an uneven polymer morphology, where a random incorporation of the L-tyrosine diphenolic monomer may have formed within the polymer backbone.
3. The acid catalysis of the polymerisation may have facilitated spontaneous depolymerisation and degradation of growing polymeric chain.
4. A extremely anhydrous polymerisation conditions (particularly dry box) are required to prepare high molecular weight polymers. Small amounts of adventitious water can disrupt the polymerisation reactions.

Although these polyacetals may find limited application e.g. drug delivery, where the PEG content of the material is desirable. For our aim, these material properties are not satisfactory. There is an underlying synthetic problem of preparing these acid labile polyacetals using the traditional method, where it is difficult to prepare high molecular weight polymers, the reason being as outlined above. In addition, these polyacetals have a low mechanical property that limits its potential processibility capabilities. The degradable element (acetal) of these polyacetals are formed during the polymerisation reactions, which is a major limiting factor of preparing acid labile polymers through this traditional method. Mainly due to the polymeric growing chain breakages due to the sensitive degradable acetal bonds degrading and breaking the growing polymer chain. Furthermore, the polyacetals **13a** and **13b** (Scheme 8) degradation yields acetals, that further degrades to aldehydes, which are potentially toxic. Although polyacetals have associated difficult biocompatibility issues, they have attracted research interests, due to these polymers undergoing rapid degradation in acidic environments [79]. Therefore, a new synthetic method of preparing acid labile polymers based on the polyacetals or similar polymers needs to be considered. The consideration must avoid toxic or potentially toxic degradation products. These new polymers need to addresses the synthetic limitation of preparing the polyacetals **13a** or **13b** using the existing method of preparing acid labile polymers.

# Chapter 3

## *Design & synthesis of an orthoester monomer*

# Chapter 3

## *Design & synthesis of an orthoester monomer*

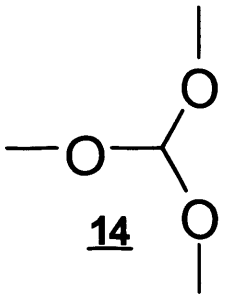
### *1.0 Introduction: orthoester monomer design and specifications*

As outlined previously with polyacetals work in chapter 2 the synthetic difficulties associated with preparing highly degradable polymers using the traditional method. This method harboured some synthetic challenges, as the degradable element of the polymer is formed in the polymerisation step. We seek to depart from the traditional method of preparing the highly degradable element of the polymer (e.g. polyacetals or poly(ortho esters)) in the polymerisation step, and find an alternative method of introducing the highly degradable element into the polymer. Furthermore, the polyacetals prepared in chapter 2 degrade to acetals, which further degrade to aldehyde that are known to be potentially toxic[79;79;80]. Therefore, we concentrated in preparing poly(ortho esters), which yields degradation products that are highly water soluble and easily washed out of the body, providing a better chance of biocompatibility of these polymers.

When designing and preparing a new orthoester monomer, high acid liability, solid material characteristics, stability, the number of synthetic steps and biocompatibility need to be taken into consideration. The monomer design needs to be based on naturally occurring metabolites, or derivatives of natural occurring chemical compounds to enhance the chance of biocompatibility.

A orthoester bonds are made of three oxygen atoms bonded to one carbon atom **14**, producing a high tendency for the bonds to come apart in slightly acidic conditions (Figure 10). A review by Dewolfe showed the different ways of preparing orthoesters that are used in protective group chemistry[81]. Orthoesters are widely used in protecting group chemistry to protect carboxylic acid groups, due to the

orthoester having high stability towards strong base and nucleophiles[81;82]. A more recent edition of Greene and Wuts protecting group chemistry shows a broad overview of different orthoesters used as protecting groups [60]. We have been focused in preparing a stable orthoester monomer that possesses an orthoester bond e.g. molecule **14** (Figure 10).

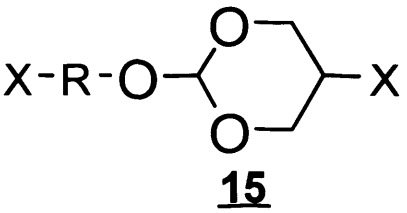


**14**

**Figure 10**: An example of an orthoester bond **14** [81].

## *1.1 Non-Bridged Orthoester Monomer Molecule Synthetic Design Requirements*

The intent is to have the orthoester monomer molecule is designed to have the orthoester degradable element embedded within the monomer, while possessing polymerisible functionality within the monomer **15** (Figure 11). This design will produce the poly(ortho esters) when polymerised with the degradable orthoester element within the monomer, rather then brought in the polymerisation step using the traditional method. The traditional approached taken to prepare poly(ortho esters) are highlighted in chapter 1.



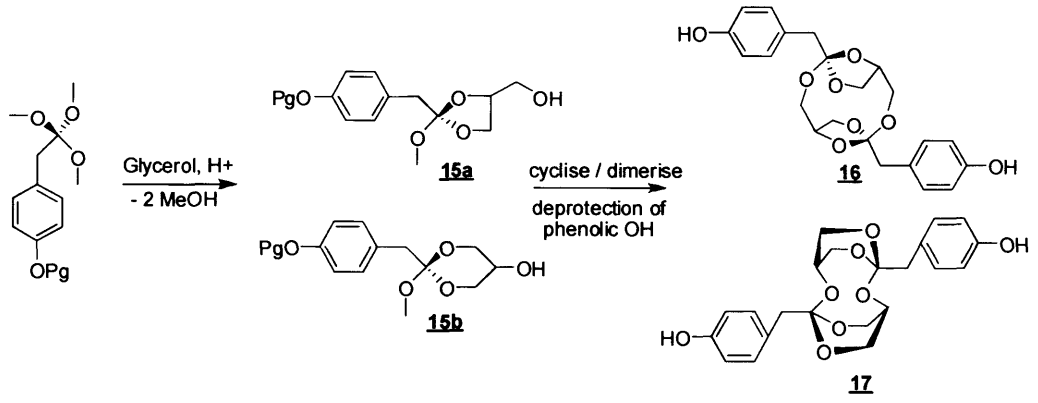

**15**

**Figure 11**: Representative orthoester monomer **15** design requirements (X represents the polymerisible functionality).

Greene and Wuts 'protective groups in organic synthesis' edition have suggested that simple orthoesters derived from normal alcohols are the least stable in terms of acid liability and stability towards strong nucleophiles [60]. However, as the orthoesters become more constrained, the stability increases. These constrained orthoesters have found wide applications due to their ease of handling and manageable reaction conditions requirements. However, if they are placed in certain conditions particularly slightly acidic environment the orthoesters are cleaved[81].

Orthoester monomer **16** and **17** were designed (Scheme 9). Orthoester monomer **16** and **17** match the design specification in the following areas:

- A limited number of four synthetic reactions steps are involved.
- The monomer is derived from the naturally occurring metabolite 4-hydroxy (phenyl acetic) acid, we believe this may increases the monomer propensity to be potentially become biocompatible.
- The constrained orthoester ring arrangement may produce a monomer that is stable.
- These stability characteristics can potentially be transferred from the monomer molecule to the macromolecule, producing a macromolecule with good processibility characteristics.

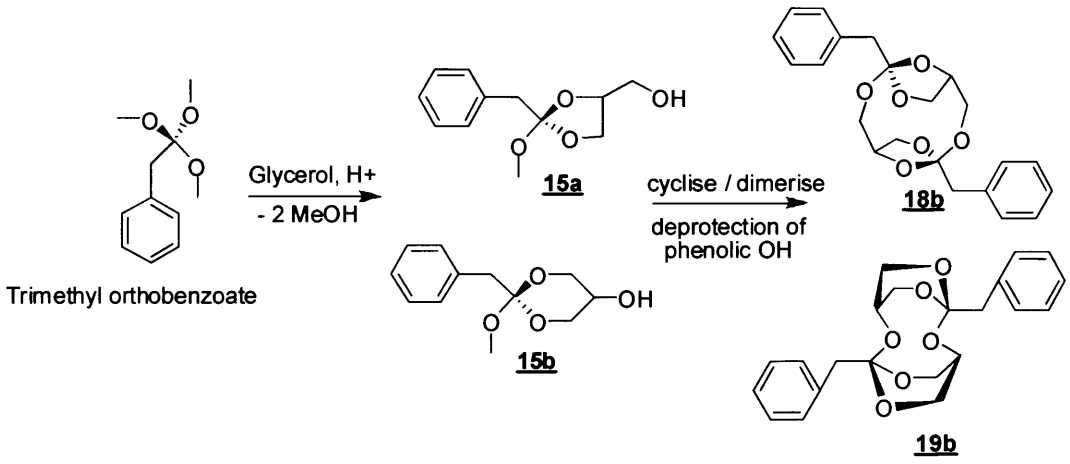



**Scheme 9**: Phenyl acetic acid & glycerol derived orthoester monomer synthesis **16** and **17**.
[Pg = protecting group]

# 2.0 *Results and Discussions*

## *2.1.1 4-Hydroxy (phenyl acetic) acid orthoester monomer synthesis*

A commercially available orthoester ester, trimethyl orthobenzoate (Sigma-Aldrich, UK) was used a starting material in the synthesis of the model isomers **18b** and **19b** (Scheme 10), a derivative of orthoester monomer **16** and **17** (Scheme 9). The aim was to understand the chemistry involved. In addition, there were no literature reports of these isomers **18b** and **19b** (Scheme 10) being produced or any information on the stability behaviour of these isomers.




**Scheme 10**: Synthesis of model orthoester isomers **18b** and **19b** prepared to investigate the stability of the orthoester ring arrangement.

An acid catalysed (p-toluene sulphonic acid (PTSA)) trans-esterification reaction of trimethyl orthoester benzoate with glycerol produced the isomer **15b** in a low yield (34 % (combined **15a** & **15b**)). The isomer **15b** was difficult to purify and isolate with the standard small molecule synthesis purification methods, including aqueous work and column chromatography. A fractional distillation method was attempted to purify the oil like isomer **15b** without success. It was assumed that the high ring strain on the cyclic five-membered orthoester isomer **15a** produced a molecule that was unstable if it is compared to similar five-membered structures within literatures[79;83]. There was no direct literature information on the isomers (**15a** &

**15b**) with which to make a comparison. Instead, a similar cyclic orthoester five-membered ring was used as a comparison to understand the stability of the isomers (**15a** & **15b**)[81]. It was noted that these types of orthoesters were prone to degradation, due to the thermodynamically unfavourable strain on the ring. Simple orthoesters prepared from alcohols are widely accepted to be least stable and have a higher tendency for the orthoester to degrade before the molecules can be purified and analysed[84]. With this in mind the methyl ether derivative of molecules **15a** and **15b** may have added to the instability of both the orthoesters.

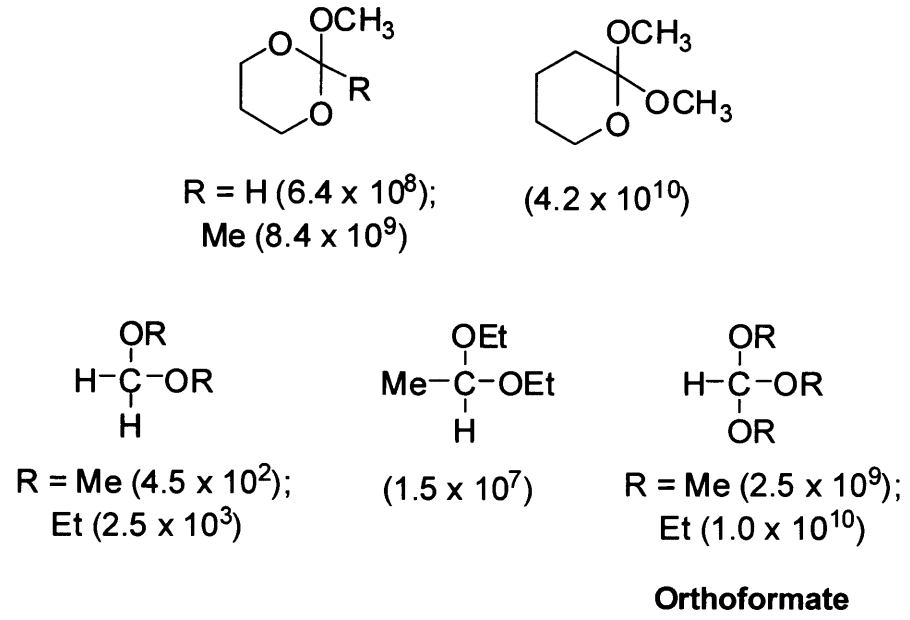



**Figure 12:** Hydrolysis rates of simple alcohol derived orthoesters [87].

### *2.1.2 Orthoester monomer molecule synthesis derived from lactones*

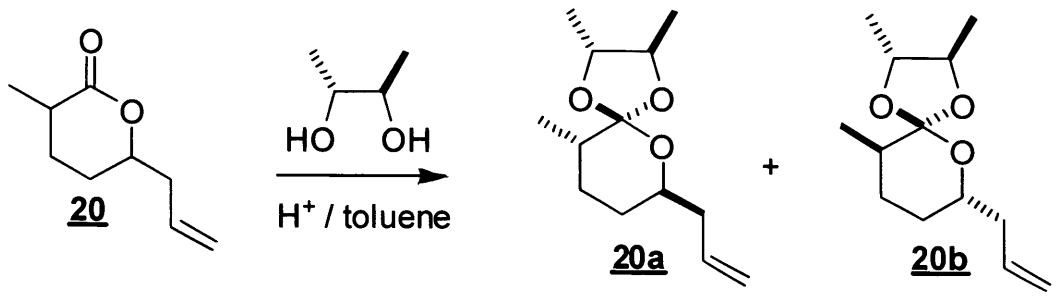
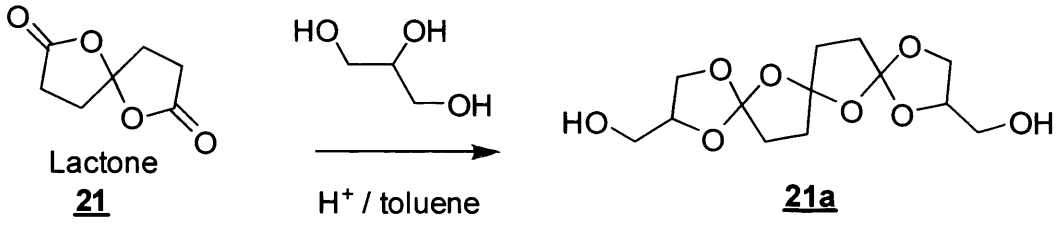



**Figure 13:** Orthoester isomers **20a** and **20b** prepared from lactone **20** and a diol[85].

Using the chemistry learned from the synthesis of orthoesters **15a** and **15b** (Scheme 10), a new rationale was developed. The aim was not to have any incorporation of simple orthoester(s) derived from alcohols within the design of the orthoester monomer. Smith et al have prepared an orthoester, where there was no simple alcohol involved to make up the orthoesters structure[86]. This concept was used to prepare an orthoester monomer molecule **21a** (Scheme 11). The idea was to address the stability limitations of an orthoester monomer molecule, which was confronted with the synthesis of orthoester **15a** and **15b** (Scheme 10). This led to a pattern emerging in that a successful orthoester monomer molecule design and synthesis required no participation of simple alcohol derived orthoester incorporated into the orthoester monomer. This may have been due to the simple alcohol derived orthoester, have a higher propensity to degrade, as they have a higher degree of instability.

Examples of simple alcohol derived orthoesters that are commercially available are the orthoformates shown in Figure 13. Orthoesters are readily hydrolysed, because the oxygen atom that makes up an orthoester group possesses two lone pair of electrons. This increases the basicity of the leaving group and facilitates hydrolysis[87]. Generally, the more hindered the carbon that bears the orthoester group is, the greater the rate of hydrolysis. For example, in a simple orthoester, if the hydrogen atom is replaced by a methoxy or even a higher alkoxy group the rate of hydrolysis increases incrementally[88;89]. Ethoxy derived orthoester molecules hydrolysed four to nine times faster then the corresponding methoxy derived orthoester. This is due to the ethoxy group being more basic and readily protonated.




**Scheme 11:** Orthoester monomer **21a** prepared by a acid catalysed reaction involving lactone **21** and a triol.

The orthoester monomer molecule **21a** (Scheme 11) was prepared with a low crude yield (12 %). It was concluded that the low yield might have been a result of the high water solubility of the molecule. This high water solubility is assumed to be due to the molecule **21a** (Scheme 11) possessing a high number of polar atoms. This therefore, led to a molar mass loss of the compound particularly in the separation, extraction and purification steps.

Molecule **21a** (Scheme 11) had a high degree of instability, although measures were put in place in the design processes to address this potential problem, by having no terminal methyl groups incorporated into the structure. From the proton nuclear magnetic resonance spectroscopy ($^1$H-NMR) there was no indication of the orthoester ring being present, after attempts were made to purify the molecule using column chromatography on silica as the stationary phase. As silica is a Lewis acid, the base triethyl amine was added to the stationary phase to increase the pH, and a 1 % triethyl amine added to the mobile phase. This had no effect on the stability of the orthoester ring, where molecule **21a** still degraded in the purification step (column chromatography).

In our search for a stable orthoester monomer, an alternative orthoester monomer molecule design was considered. This orthoester monomer molecule designed must address the problems and issues that were faced in the two-orthoester monomer synthesis that have been described (Section 2.1 and 2.1.2). The general obstacles and issues that may occur include:

- High water solubility due to an increased number of polar atoms incorporated into the molecule design. This increased mass loss in the purification steps.

- Increased instability due to simple alcohols derived orthoester incorporated into the orthoester group. This produced molecules that readily hydrolysed.

- Monomers were unsuitable for purification by conventional small molecule purification steps (e.g. column chromatography).

An alternative rationale was developed that addressed the obstacles that were encountered within section 2.1 and this new rationale is discussed in section 2.2.

## *2.2 Introduction: Bicyclic [2.2.1] orthoesters*

This section attempts to address the stability issues that were encountered previously with the acyclic based orthoester monomer synthesis, which was attempted particularly with the 4-hydroxy (phenyl acetic) acid **18b** or **19b** (Scheme 10) and the lactone **21a** (Scheme 11) derived monomer synthesis.

To address these stability issues a new rationale was developed which aimed to prepare an orthoester monomer based on cyclic orthoesters, because cyclic orthoesters are recognised to be far more stable then the acyclic orthoesters [81]. A five membered bridged bicyclic orthoester monomer molecule was investigated.

### *2.2.1 itaconic acid derived orthoester monomer synthesis*

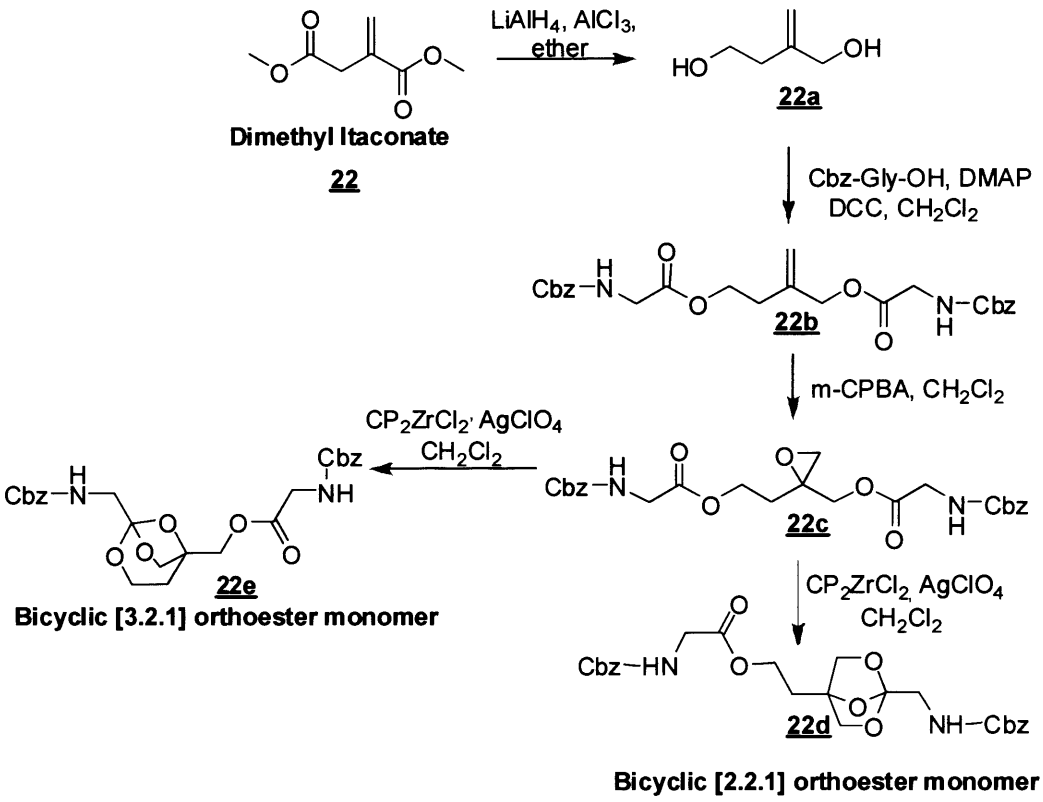
The itaconic acid derived orthoester monomer synthesis was attempted to addresses the problems previously faced with the acyclic orthoester monomer synthesis **22d** (Scheme 12). The rationale was that the monomer molecule **22d** possessed characteristics, which may address the synthetic limitation previously faced with the non-bridged acyclic orthoester monomer molecules (section 2.1). The advantageous characteristics of itaconic acid derived orthoester monomer **22d** include:

- The two hydrophobic phenyl groups in **22d** will limit molecular mass loss in the extraction phase of the purification steps. Provides a more manageable molecule to work with and purify if need be by conventional small molecule purification methods (column chromatography).

- The orthoester monomer molecule design has no simple alcohol derived orthoesters incorporated into the molecule design. It was hoped that this would increase molecular stability and limit decomposition, in particularly in the purification, general handling and storage of the compound. The design

was based on a bicyclic orthoester, which, are widely recognised to have higher stability characteristics compared to orthoester derived from simple alcohols (non-bridged orthoesters).

- Monomer **22d** design was derived from itaconic acid, which itself is a naturally occurring metabolite. The design also includes the amino acid glycine, which can potentially increase the chance of biocompability of the monomer.




**Scheme 12:** Synthesis of itaconic acid derived orthoester monomer molecule **22d** and **22e**.

In the synthesis of the orthoester monomer molecule **22d** (Scheme 12), dimethyl itaconate was considered as the starting point due to commercial availability and one synthetic step leads onto to the vinylic diol **22a** (Scheme 12). Also the precursor epoxide **22c** to the bicyclic [2.21] orthoester **22d** (Scheme 12) have be prepared with ease. Wilson et al have developed a mild method of reducing conjugated vinyl and

ester using a mixture of lithium aluminium hydrate and aluminium trichloride in a 3:1 ratio[90]. This produced an alane that reduced the diesters selectively in the presence of vinyl groups cleanly to produce **22a** (15 %). Aqueous ammonium chloride was used to neutralise the excess remaining reducing agents. Extraction with ethyl acetate three times produced a low yield, due to the high polarity characteristics of the vinylic diol **22a**.

Esterification of the vinylic diol **22a** with benzyl protected glycine. This yielded the glycine derived vinylic ester **22b** (47 %) as a crystalline material, also it should be noted the benzyl protection was removed in the final step of the synthesis. Glycine derived vinylic ester **22b** was esterified with the amino acid glycine to produce a crystalline material that is more manageable to work with, and increase the potential biocompatibility of the monomer and the polymer itself. The chance of biocompability was thought to be further increased by the incorporation of glycine. In addition to bioresorbability, glycine incorporates other advantageous characteristics such as maintaining the acid labile orthoester bonds in basic pH values, if the two free primary amines are generated (Figure 14). It was hoped this would protect the monomer from degradation from atmospheric moisture or other changes that the monomer is sensitive to.

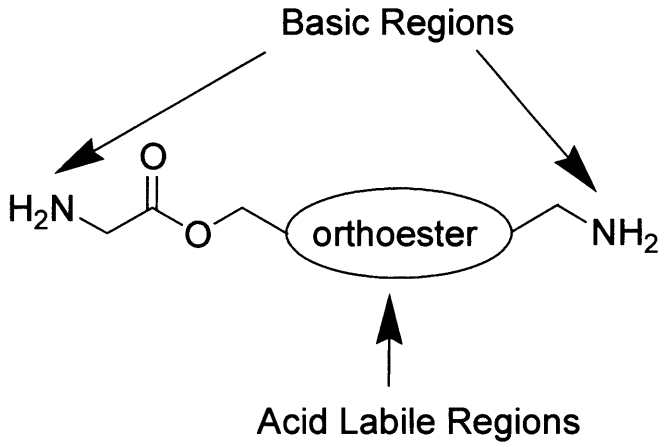



**Figure 14:** pH sensitive regions incorporated within orthoester monomer design with terminal primary amino groups to keep the molecule from atmospheric led degradation.

Esterification was achieved by the use of dicyclohexyl carbodiimide as the carbodiimide coupling method, in the presence of a catalytic amount of dimethylaminopyridine (DMAP). The vinylic ester **22b** was purified by extraction

work-up and column chromatography on silica. It was rationalised that the use of benzyl-protected glycine (the benzyl protected groups will be removed in the final synthetic step), the phenyl groups within the molecular structure would decrease the hydrophilicity. However, experimentally the molecule polarity could not be sufficiently decreased to achieve yield greater then 80 %. The glycine derived diester **22b** was oxidised with *m*-chloroperoxy benzoic acid to prepare the epoxy glycine diester **22c** (42. 3%). Mass loss behaviour of molecule **22b** due to the hydrophilic nature of the molecule was found with molecule **22c**.

Zirconocene catalysed epoxy ester rearrangement reaction on molecule **22c** yielded a mixture of products. One of the mixture being the bicyclic [2.2.1] glycine derived orthoester **22d** (12 %) and another being the bicyclic [3.2.1] glycine derived orthoester **22e** (Scheme 12) was prepared. The itaconic acid derived bicyclic [2.2.1] orthoester **22d** through zirconocene catalyzed rearrangement reaction were very unstable. There was no evidence that the desired bicyclic [2.2.1] orthoester **22d** or **22i** was prepared, instead an acetal **22h** was observed (Figure 15). It was assumed the orthoester ring arrangement produced a molecule with a high strain. This produced an unstable orthoester molecule, which degraded rapidly in the $^1$H-NMR analysis ($CDCl_3$), as the orthoester signal 3.9 – 4.05 ppm disappeared quickly on repeat NMR run. Also, it was difficult to know which orthoester was potentially prepared. This was due to the symmetry of the molecule, where both side of the molecule from the reactive epoxide was asymmetric. However, there was not enough supportive information to assume if the reaction did not work.

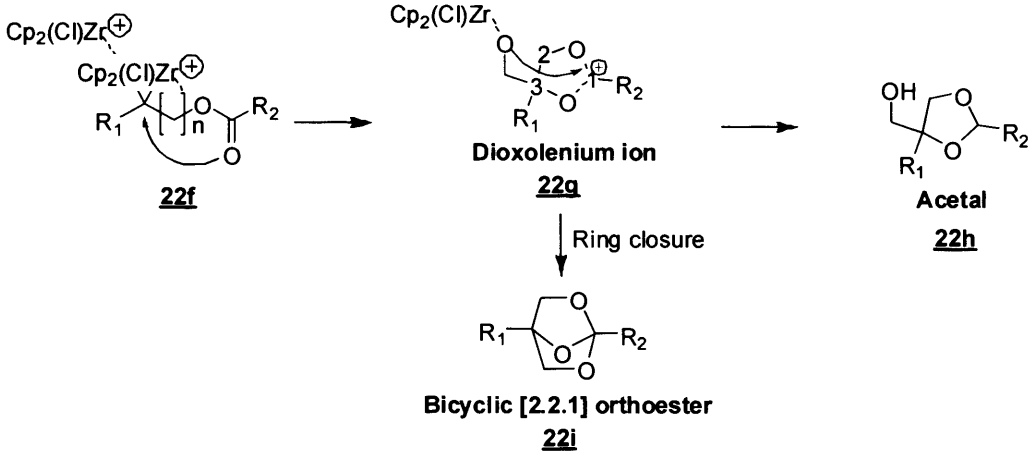
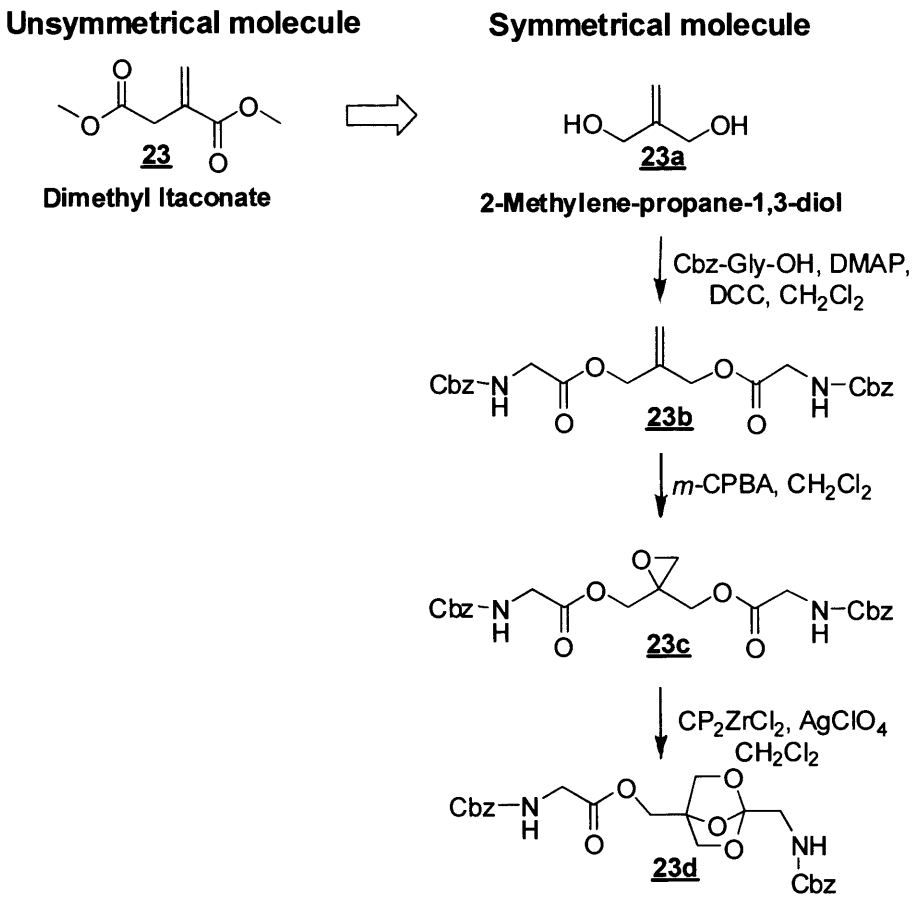



**Figure 15:** Zirconocene catalyzed epoxy ester-orthoester **22i** rearrangement mechanism[91].

The mechanism involved as suggested by Wipf et al include formation of a cationic zirconocene **22f**, followed by neighbouring-group assisted Lewis-acid induced opening of the epoxide to the dioxolenium ion **22g** (Figure 15)[91]. Irreversible $S_N2$-attack of the zirconocene-complexed alkoxide at position 1 of dioxolenium ion leads to the bicyclic orthoester products **22i**, depending on the number of carbons between the ester group and the reactive epoxide group (Figure 15).

### *2.2.2 2-Methylene-propane-1,3-diol derived orthoester monomer molecule synthesis*

To address the symmetry problems of the itaconic acid derived orthoester monomer molecule design and synthesis, the monomer molecule design was rationally adapted. The design aim was to produce a symmetrical precursor molecule **23c** prior to the orthoester reaction (Scheme 13). It was hoped this would elevate the associated mixture of bicyclic orthoesters products formation and assignment of product formation (Scheme 12). Because through the symmetrical 2-methylene-propane-1,3-diol **23a** only one possible orthoester product formation was possible, this being the bicyclic [2.2.1] orthoester **23d** (Scheme 13).




**Scheme 13:** Synthesis of 2-Methylene-1,3-propane diol **23a** derived orthoester monomer molecule **23d** [92].

2-Methylene-1,3-propanediol **23a** (Scheme 13) was used as the starting compound due to the commercial availability and symmetrical arrangement of the molecule. The molecule was diesterified with Cbz protected glycine using dicyclohexyl carbodiimide, as the coupling agent. This produced a crystalline compound **23b** ( 87 %) (Scheme 13). An epoxide was generated from the terminal methylene group of compound **23b** using *m*-Chloroperoxybenzoic acid, which lead to the precursor orthoester molecule **23c** (79 %) being prepared as a white crystalline material. The same reaction condition previously used with itaconic acid derived orthoester monomer synthesis was used with this molecule. Zirconocene catalyzed rearrangement reaction on the precursor orthoester **23c** did not produce the desired bicyclic [2.2.1] orthoester **23d** (Scheme 13). An acetal was formed instead (Figure 15) and this confirmed that no bicyclic [2.2.1] orthoester was formed in the itaconic

acid derived orthoester synthesis (reactions involving molecule **22c** to **22d** (Scheme 12). Therefore, the orthoester that was prepared was the bicyclic [3.2.1] orthoester **22d** (Scheme 12), and the remaining mixtures of impurities could have reaction side products.

### *2.2.3 Study of different catalyst effects on the formation of bicyclic [2.2.1] orthoester monomer*

Synthesis of an orthoester monomer molecule through the itaconic acid and 2-methylene-1,3-propanediol synthetic routes using zirconocene as the catalyst produced the following results:

1. The number of carbon atoms present in between the reactive epoxide ring and ester group determines the types of orthoester that are prepared. For example, if there are two carbon atoms present then a bicyclic [3.2.1] orthoester was formed. Moreover, if there is one carbon atom then no bicyclic [2.2.1] orthoester was prepared.

2. The symmetry of the molecule was also recognised as an important factor, especially if there are two or more esters groups away from the reactive epoxide group.

Although zirconocene was used as the catalyst in the synthesis of the desired bicyclic [2.2.1] orthoester synthetic studies, it was important to understand the significance of the catalyst(s) involved in the rearrangement reaction to form the bicyclic [2.2.1] orthoester. Within the literature, other research groups have prepared similar bicyclic [2.2.1] orthoesters using Lewis acid catalyzed epoxy ester-orthoester rearrangement reactions[93-96]. Giner et al have shown a similar method of preparing the bicyclic [2.2.1] orthoester, where an oxetane ring replaced the epoxide (Figure 16). The reaction mechanism was similar to the mechanism as shown previously (Figure 15) apart from the use of a bronsted acid (TFA) as the catalyst.

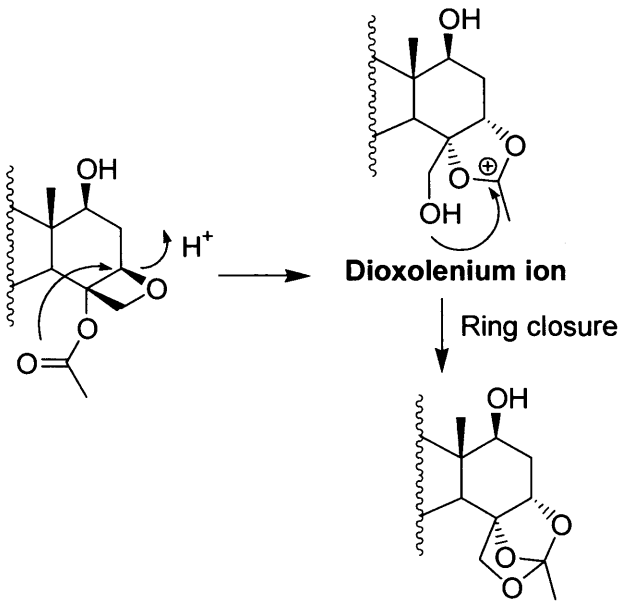



**Figure 16:** Mechanism of Bronsted acid catalyzed bicyclic [2.2.1] orthoester rearrangement reaction [97].

Epoxide **23c** (Scheme 13) was treated with catalytic amount of TFA at ≈ 20 °C overnight in anhydrous $CH_2Cl_2$. The reaction prepared the desired bicyclic [2.2.1] orthoester **23d** (Scheme 13) (32 %). Orthoester **23d** was unstable and degraded in the $^1$H-NMR analysis stage. This may have been due to the constrained five membered ring arrangement of the bicyclic [2.2.1] orthoester, producing a thermodynamically unstable molecule. Also, the presence of the acid catalyst TFA may have assisted degradation. To control the reaction and minimise degradation, one further experiment was conducted at – 18 °C, using the same reaction conditions as before. This had no effect on the stability of the orthoester; however, the rate of reaction was decreased.

### *2.2.3.1 Monomer molecule generated from bicyclic [2.2.1] orthoester molecule.*

Attempts were made to deprotect orthoester **23d** (Scheme 13) to generate the amino acid glycine derived bicyclic [2.2.1] orthoester monomer molecule (Figure 17). The deprotection of the two Cbz groups of orthoester **23d** to generate the two free amines was prepared successfully **23e** (Figure 17) (98 % (calculated by $^1$H-NMR)). The deprotection was achieved using 5 % palladium on activated charcoal as the catalyst

and hydrogen gas in absolute ethanol. The orthoester **23d** degraded in the deprotection step and generated the more stable acetal **23f** (Figure 17). It was difficult to assign the orthoester degradation to deprotection steps, as degradation occurred before in the orthoester formation. The aim of the deprotection reactions was to get an insight into the potential side reaction occurring. This side reaction may occur between the nucleophilic free amines and the esters or some other side reaction. From the deprotection experiments, there was no evidence of a side reaction occurring.

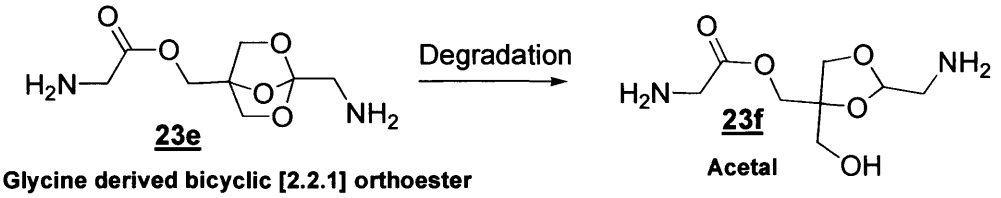



**Figure 17:** Glycine derived bicyclic [2.2.1] orthoester **23e** and degradation acetal **23f** product.

There still remains synthetic difficulties particularly for the orthoester monomers that have been examined. For an orthoester monomer design and synthesis to be successful, the following issues faced in this chapter needs to be addressed:

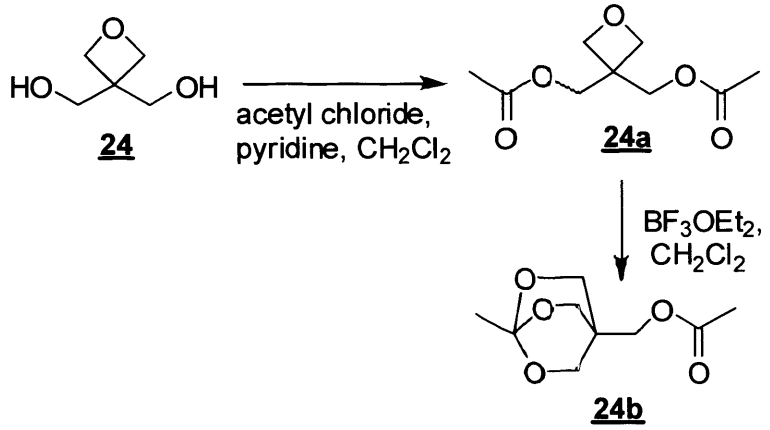
- The instability of the five membered ring arrangement of the orthoester ring, producing a molecule with a high ring constraint and a high thermodynamic instability. This produced a molecule that readily hydrolysed.

The alternative rationale and strategy that was employed to addresses the problematic issues faced in preparing a five membered bicyclic [2.2.1] orthoester monomer. One possibility was to increase the size of the ring of the molecular structure, which may increase the stability of the molecule.

## *2.3 Introduction: Bicyclic [2.2.2] orthoesters*

This section is concerned with addressing the stability issues that were faced in the synthesis of bicyclic [2.2.1] orthoester monomer molecule **22d** and **23d** (Scheme 12 and 13). To address these stability issues alternative orthoester(s) with a high

stability were investigated. An example includes the bicyclic [2.2.2] orthoester **24b** (Scheme 14). The rationale was that a six membered orthoester ring arrangement of the bicyclic [2.2.2] orthoester **24b** (Scheme 14) may increase the stability of a monomer. Largely, due to an increase of the number of carbons in the orthoester ring arrangement, thus producing an orthoester ring that is under less strain and has a lower thermodynamic energy. Therefore, it is hoped that this ring arrangement may reduce the rate of hydrolysis and increase stability.




**Scheme 14:** Synthesis of model bicyclic [2.2.2] orthoester **24b** derived from oxetane diol **24**.

Bicyclic [2.2.2] orthoesters have found wide use in protecting group chemistry, as a result of their stability. These types of orthoesters have often been prepared from nitriles or imido esters, or by orthoester exchange reactions. However, there were no examples of preparing orthoesters directly from carboxylic acids, until the example of Corey and Raju, who prepared bridged bicyclic [2.2.2] orthoesters from carboxylic acids[98]. These types of bridged bicyclic [2.2.2] orthoesters derived from 2,2-bishydroxymethyl-1-propanol were especially useful because of their chromatographic stability as compared to acyclic orthoesters [98;99]. As a result of the chromatographic stability of bicyclic [2.2.2] orthoesters and the ease by which they could be separated and purified, these orthoesters have found wide application in the field of protecting group chemistry [60;100].

Although bicyclic [2.2.2] orthoesters have been routinely used in protecting group chemistry and their chemistry is well known, there are no examples in literature of

their use in orthoester monomer synthesis. Where the orthoesters are incorporated into a monomer, which is then polymerised to prepare poly(ortho esters) *co*-polymers. Here we report new examples of bridged bicyclic [2.2.2] orthoesters that are used to prepare a first generation of poly(ortho esters) *co*-polymers.

### *2.3.1 Model bicyclic [2.2.2] orthoester derived from acetyl chloride*

Within the literature, little is known about the chemistry involved in the design and synthesis of a bicyclic [2.2.2] orthoester monomer. Therefore, it was important to develop a model orthoester synthesis (Scheme 14) to understand the following:

1. Does the oxetane ester rearrangement reaction proceed on a symmetrical molecule **24** (Scheme 14), where there are two reactive ester functional groups present.
2. The rearrangement reactions are catalysed by the Lewis acid $BF_3$ etherate, therefore will the present Lewis acid degrade the orthoester when formed?

Pentaerythritol monobromide undergoes a $S_N2$ reaction in the presence of potassium hydroxide to produce the oxetane diol 3,3-bis (hydroxymethyl) oxetane **24** (63 %) as a clear oil[101]. The oxetane diol **24** was purified via vacuum distillation (0.5 mm / Hg) at 180 °C to produce a semi-sold hygroscopic material. The $^1$H-NMR of oxetane diol **24** is shown in Figure 18.

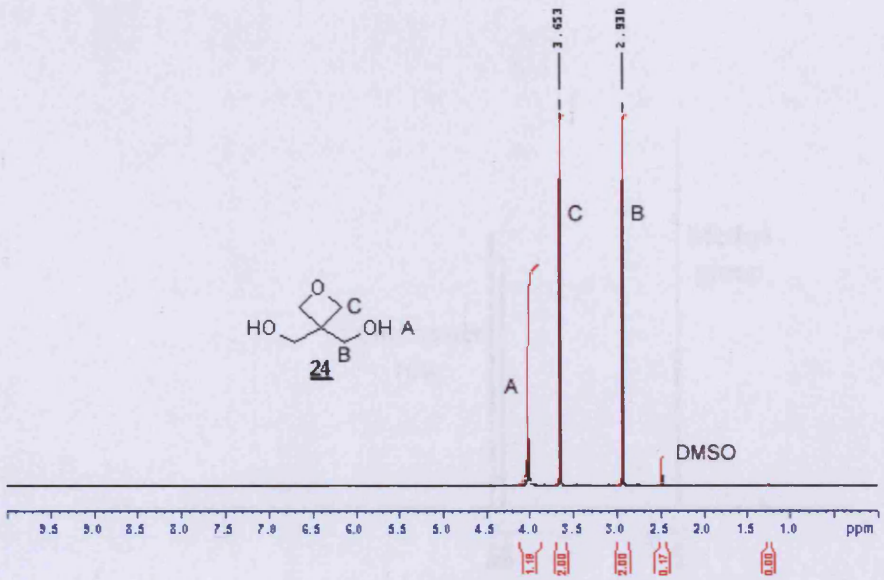



**Figure 18:** $^{1}$H-NMR spectrum of oxetane diol **24**.

The oxetane diol **24** was diesterified using acetyl chloride in basic conditions (triethyl amine) overnight at room temperature. The reaction produced **24a** (62 %) as brown oil. The low yield was attributed to mass loss in the extraction and purification phase, due to high polarity of the molecule. Purified **24a** was dissolved in anhydrous $CH_2Cl_2$ and catalytic amount of $BF_3$ etherate (3 mol %) in a diluted $CH_2Cl_2$ solution was injected dropwise under a flow of argon. At all times the reaction was strictly kept under an anhydrous atmosphere to prepare the bicyclic orthoester **24b** (80 %) as a white solid. The $^{1}$H-NMR of molecule **24b** is shown in Figure 19.

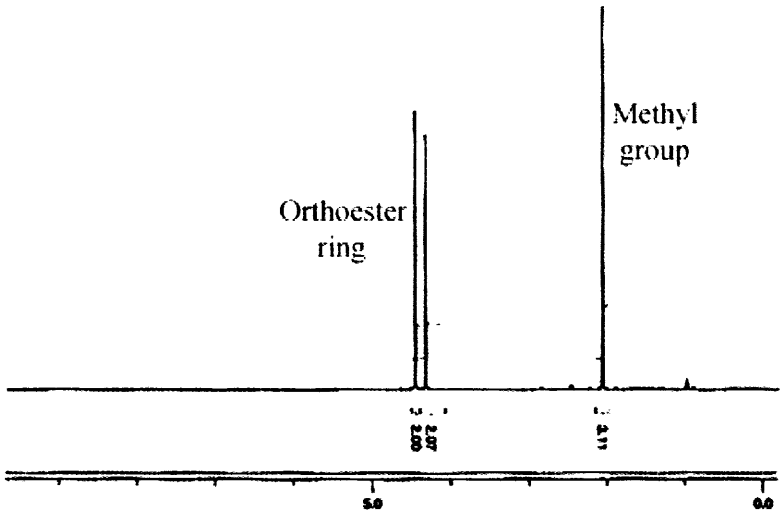



**Figure 19:** $^{1}$H-NMR spectrum of bicyclic orthoester **24b** derived from acetyl chloride.

Through the model bicyclic [2.2.2] orthoester synthesis derived from acetyl chloride, we report a first example of bicyclic [2.2.2] orthoester being prepared from a symmetrical molecule. In addition, we observed the rearrangement reaction which seems to satisfactorily proceed in the presence of competitive reactive species (Figure 20).

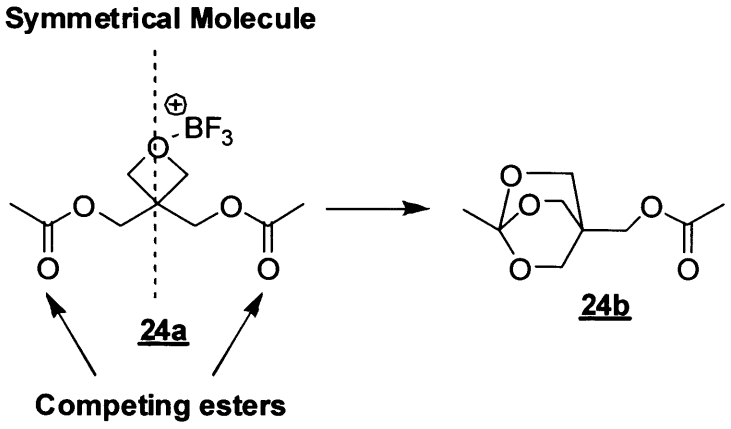



**Figure 20:** Bicyclic [2.2.2] orthoester **24b** formation in a symmetrical molecule **24a**.

The model orthoester synthesis has shown the chemistry has satisfied our aim in two ways:

1. The rearrangement reaction proceeded satisfactorily with no orthoester degradation over the 18 h during which the reaction was conducted.

2. Bicyclic [2.2.2] orthoester can be prepared as symmetrical molecules with competitive reactive species.

Bicyclic [2.2.2] orthoester **24b** (Figure 21) has shown that an increase of a carbon in the cyclic ring arrangement of an orthoester has increased the stability of orthoester over all. We assume this increase in stability behaviour may be attributed to the ring arrangement which is under less strain than the ring arrangement of the bicyclic [2.2.1] orthoester **23d** (Scheme 13) previously investigated.

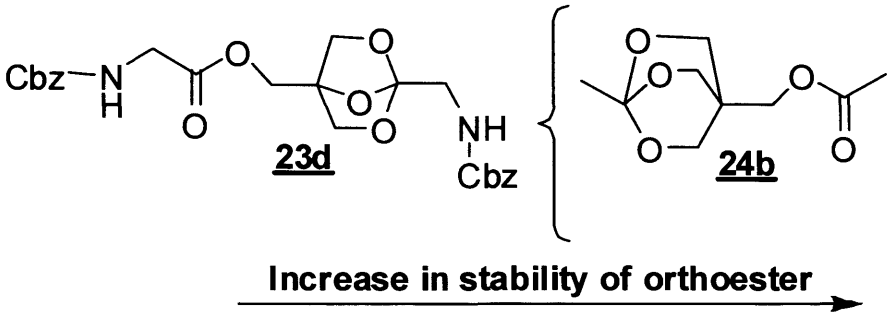
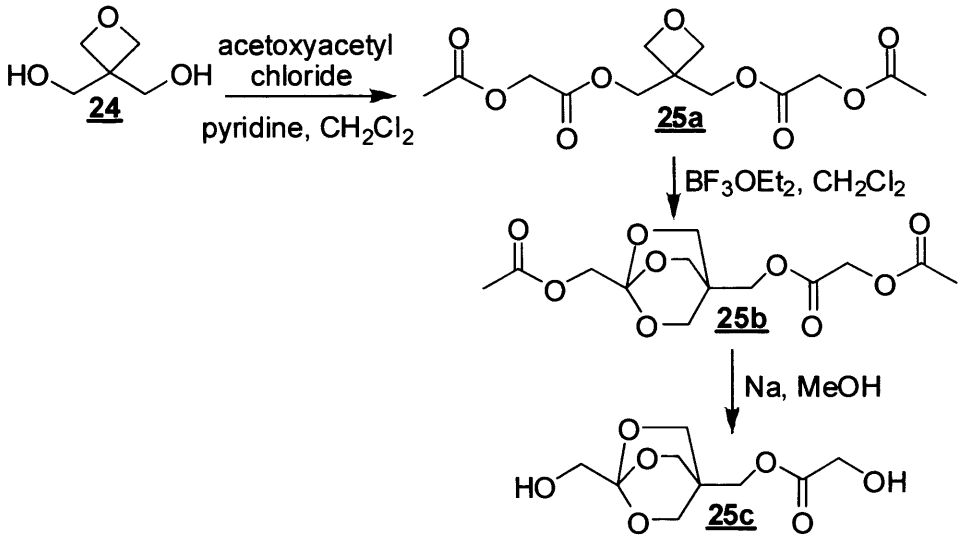



**Figure 21:** Stability behaviour of different bicyclic orthoesters **24b** and **23d**.

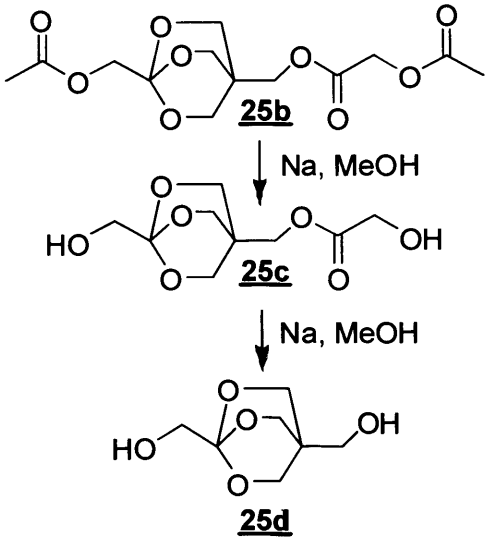
### *2.3.2 Glycolic acid derived bicyclic [2.2.2] orthoester monomer molecule synthesis*

Using the model orthoester **24b** (Scheme 14) as a template, a bicyclic [2.2.2] orthoester monomer **25c** (Scheme 15) was rationally designed and developed. The design was based on the naturally occurring metabolite glycolic acid.




**Scheme 15:** Glycolic acid derived bicyclic [2.2.2] orthoester monomer **25c** synthesis.

Oxetane diol **24** was esterified with acetoxyacetyl chloride using the same reaction conditions as reaction of **24a** in anhydrous THF. The reaction prepared oxetane diester **25a** (43 %) (Scheme 15) as a clear oil. As previously observed with the precursor model orthoester **24a** (Scheme 14) mass loss also occurred in the purification phase, due to the high polarity nature of the molecules. The oxetane diester **25a** (Scheme 15) under went the same orthoester rearrangement reaction conditions as previously used with molecule **24b** (Scheme 14), which prepared the bicyclic [2.2.2] orthoester **25b** ( 78 %). To reduce mass loss, the molecule **25b** was directly purified by being chromatogramed on silica with 1 % triethylamine in the mobile phase, which produced a white crystalline material. The compound was stored in a completely anhydrous atmosphere to reduce the propensity of degradation. The acetate protecting groups of glycolic acid alcohols, were cleaved using sodium methoxide in methanol to produce a mixture of monomers. These included; the bicyclic [2.2.2] orthoester monomer molecule **25c** (Scheme 16) (13 %) and bicyclic orthoester monomer molecule **25d** (Scheme 16) (12 %). Both products were oils. Molecule **25d** was a further cleavage of the glycolic ester of molecule **25c**. This cleavage may have been limited or completely inhibited by the use of selective reagents that cleave acetate protecting groups[102-104]. However, due to the molecular structure of molecule **25c** and **25d** producing molecules that have a high polarity, mass loss would remain high in the purification steps.




**Scheme 16:** Bicyclic [2.2.2] orthoester monomer diol **25d** prepared from bicyclic [2.2.2] orthoester monomer **25c**.

The acid chloride derived model bicyclic [2.2.2] orthoester (Scheme 13) and glycolic acid derived bicyclic [2.2.2] orthoesters (Scheme 15 & 16) pathways have produced the following results:

1. A stable orthoester can be produced from the bicyclic [2.2.2] orthoesters, that has evolved from the orthoester monomer synthetic strategies previously undertaken (section 2.1 and 2.3).

2. A stable orthoester monomer molecule was prepared, which was a derivative of natural metabolites (glycolic acids).

We have for the first shown that bicyclic [2.2.2] orthoesters can be incorporated into a monomer design and synthesis and are derived from natural occurring metabolites. However, there remains a synthetic issue of low monomer yields **25c** & **25d** (Scheme 15 & 16), which needs to be addressed. In order, to tackle the high mass loss of both molecules **25c** & **25d**, the synthetic routes (Scheme 15 & 16) and molecule design need to be rationally adapted. While maintaining the required chemical functionality for the oxetane ester rearrangement reaction to proceed **25a** (Figure 22). The highly hydrophobic characteristics and the required chemical functionality for a new rationally adopted bicyclic [2.2.2] orthoester monomer

synthesis is shown in Figure 22. Which attempts to improve on the synthetic design of the glycolic acid derived bicyclic [2.2.2] orthoester monomer molecule synthesis previously investigated **25c** (Scheme 15).

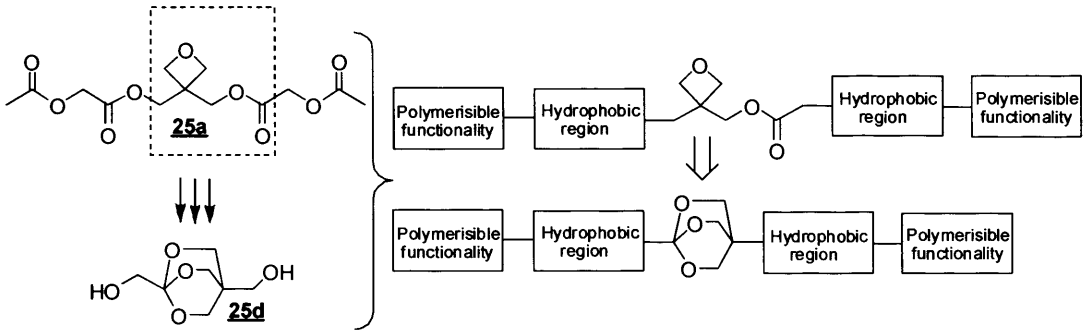


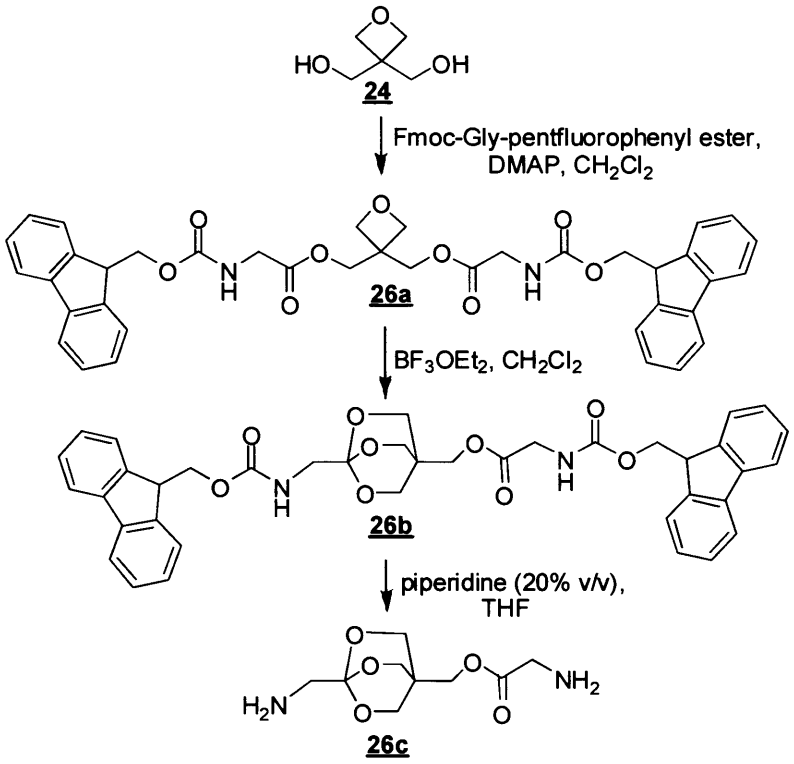

**Figure 22:** Chemical functionality and structural requirements for bicyclic [2.2.2] orthoester rearrangement reaction that is needed for monomer synthetic design of **25d**.

### *2.3.3 L-glycine acid derived bicyclic [2.2.2] orthoester monomer synthesis*

The amino acid derived bicyclic [2.2.2] orthoester monomer molecule was designed using the glycolic acid derived bicyclic [2.2.2] orthoester monomer molecule as a template. In addition, the design incorporated the advantageous characteristics identified in Figure 22. The amino acid glycine is recognised as one essential amino acid, which is used in the preparation of proteins. From a design perceptive this produced a greater tendency for the monomer to posses bioresorbable characteristics. Glycine adds advantageous features and characteristics previously identified and developed from the investigation of itaconic acid derived bicyclic [2.2.1] orthoester monomer synthesis (section 2.2). The features and advantageous characteristics include:

- Glycine deprotection produces primarily amines, which maintain the acid labile orthoester bonds in a basic environment (Figure 14). This reduces potential degradation.

- It was assumed that the glycine may produce a solid crystalline material, which can be handled with ease.




**Scheme 17:** Glycine derived bicyclic [2.2.2] orthoester monomer **26c** synthesis.

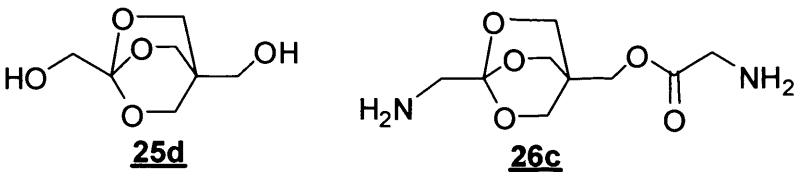
Oxetane diol **24** was esterified using Fmoc protected glycine to produce molecule **26a** (82 %) as a white powder material (Scheme 17). The diesterification was achieved using the coupling agents dicyclohexyl carbodiimide and catalytic amounts of DMAP in anhydrous $CH_2Cl_2$. The oxetane diester **26a** was purified by conventional small organic molecule purification (washing, and extraction). The crude material was chromatogramed with silica to remove the by product dicyclohexyl urea and small amounts of the unwanted mono esterified product.

The oxetane diester **26a** was dissolved in anhydrous $CH_2Cl_2$ and a catalytic amount of $BF_3$ etherate (3 mol %) in a diluted $CH_2Cl_2$ solution was injected dropwise under a flow of argon. At all times the reaction was kept strictly under an anhydrous atmosphere to produce the glycine derived bicyclic orthoester **26b** (90 %) as a white solid. The crude product was purified directly using column chromatography on pre-treated silica with 1 % triethylamine, and the mobile phase with 1 % triethylamine to maintain the pH of the chromatography system at a neutral pH. The glycine derived

bicyclic [2.2.2] orthoester **26b** was stable and could be easily handled in the laboratory.

The Fmoc protecting group of the glycine derived bicyclic [2.2.2] orthoester **26b** was cleaved under mild basic conditions. The standard cleavage reagents and conditions used to remove the Fmoc group, was a solution of 20 % piperidine in DMF stirred overnight[105]. This condition degraded the orthoester and simultaneously cleaved the Fmoc group. To understand the degradation, a $^1$H-NMR experiment was conducted on molecule **26b** with 20 % piperidine in deuterated $^1$H-NMR solvent ($CDCl_3$). This resulted in no orthoester degrading or cleavage occurring. However, a small amount of Fmoc cleavage was observed. Therefore, Fmoc protected orthoester **26b** was dissolved in a solution of 20 % piperidine and anhydrous THF. This produced the bicyclic [2.2.2] orthoester monomer molecule **26c** (18 %). The mass loss occurred due to the high polarity of the molecule, where mass was lost in the purification steps (extractions and washing).

The glycine derived bicyclic [2.2.2] orthoesters monomer molecule **26c** was designed to possess hydrophobic characteristics, to limit mass loss in the purification (Figure 23). The monomer design was based on the glycolic acid derived bicyclic [2.2.2] orthoester monomer, where the glycine derived monomer was rationally designed to incorporate the high water solubility of monomer **25d** (Scheme 16). It seems the monomer molecule needed greater hydrophobic characteristics than molecule **26c** to decrease mass loss during the purification step.



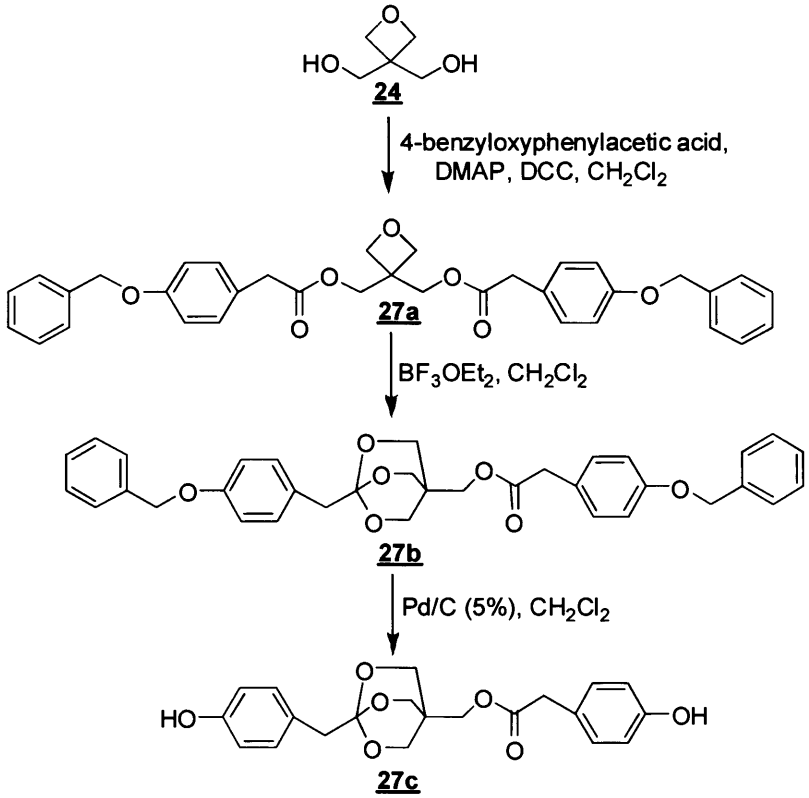

**25d** **26c**

**Figure 23:** Examples of bicyclic [2.2.2] orthoester monomer **25d** with hydroxy functionality for polymerisation and **26c** with amine for polymerisation.

Although we have shown synthetic feasibility of producing both glycine and glycolic acid derived bicyclic [2.2.2] orthoester monomer molecules (**25d** & **26c**), that are

stable enough to be handled in the laboratory, there remains an issue in the design and synthesis that needs addressing. The main issue is the large mass loss in the final step of the monomer synthesis. This results in molecules that are small in structure and have a high polarity that undergo mass loss in purification steps (extraction and washing).

### *2.3.4 Phenyl acetic acid derived bicyclic [2.2.2] orthoester monomer synthesis*




**Scheme 18:** Phenyl acetic acid derived bicyclic [2.2.2] orthoester monomer **27c** synthesis.

The phenyl acetic acid derived bicyclic [2.2.2] orthoester monomer molecule design template was based on the monomer **26c** (Scheme 17). The design rationale was to improve the synthetic yield of the final bicyclic [2.2.2] orthoester monomer molecule in the synthetic route (Scheme 18). Improved yield of monomer **27c** (Scheme 18) was designed in such a way that the two hydroxy phenyl functional groups in the molecular structure would decrease mass loss in the purification step, by increasing hydrophobicity of the molecule. A reduction in the polarity of monomer molecule **27c** and an increase in molecular size, would reduce the likelihood of molecules

sticking to the silica column (column chromatography) in purification step. Therefore, increasing the final monomer yield. Incorporation of phenyl acetic acid into the monomer design increases the tendency for the monomer to be bioresorbable, as phenyl acetic acid is a naturally occurring metabolite and is found widely in nature.

Phenyl acetic diester **27a** was prepared by esterification between oxetane diol **24** and commercially available phenyl acetic acid (Sigma-Aldrich, UK), using dicyclohexyl carbodiimide and catalytic amount of DMAP (Scheme 18). The esterification produced phenyl acetic diester **27a** as a white crystalline material (91 %). The crude product was purified by extraction, then column chromatographed on silica. The phenyl acetic diester **27a** was dissolved in anhydrous $CH_2Cl_2$ and catalytic amounts of $BF_3$ etherate (3 mol %) in a dilute $CH_2Cl_2$ solution was injected dropwise under a flow of argon. The reaction was stirred at ambient temperature overnight (18 h), then neutralized by the addition of triethylamine, filtered through celite®, concentrated and purified by column chromatography on silica. The chromatography was conducted on pre-treated silica with 1 % triethylamine in both the stationary and the mobile phase. This prepared the phenyl acetic acid derived bicyclic [2.2.2] orthoester **27b**, as a white crystalline material (65 %). The orthoester molecule **27b** was prepared as analytically pure and no further purification was required.

The protecting phenyl groups of the bicyclic [2.2.2] orthoester **27b** were cleaved using standard hydrogenation conditions[100], which involved dissolving the bicyclic [2.2.2] orthoester **27b** in absolute ethanol, and catalytic amounts of palladium on activated charcoal (10 % w/w) was added. The reaction was kept under hydrogen gas overnight while stirring at ambient temperature. The hydrogenation produced the phenyl acetic acid derived bicyclic [2.2.2] orthoester monomer **27c** (Scheme 18) (85 %). The by-products of the reaction included toluene and a small amount of mono phenyl ether (protecting group of phenyl hydroxy group). The hydrogenation as the cleavage method for removing the protecting groups prepared orthoester monomer **27c** that does not require aqueous work-up or extraction. The crude product was obtained by filtration through celite® and concentrated in vacuo. The crude product, orthoester monomer **27c** was directly purified on silica with 1 % triethylamine in both stationary and mobile phase to prepare the monomer in high yield and

crystalline material. The orthoester monomer **27c** (Scheme 18) was easy to handle and stable enough to store in a completely anhydrous environment.

The orthoester monomer **27c** (Scheme 18) is the first example of the preparation of a potentially highly degradable and sensitive orthoester monomer that is stable and can be prepared at high yield. These better matches the orthoester monomer specifications outlined previously (Chapter 1: aims). The monomer molecule **27c** was designed and synthesised to be co-polymerised with commercially available monomers, therefore, producing a new first generation poly(ortho esters) *co*-polymers. These *co*-polymers attempt to address the synthetic limitation in producing poly(ortho esters) *co*-polymers using the current traditional methods.

### *2.3.5 Rationale evolution of monomer synthesis.*

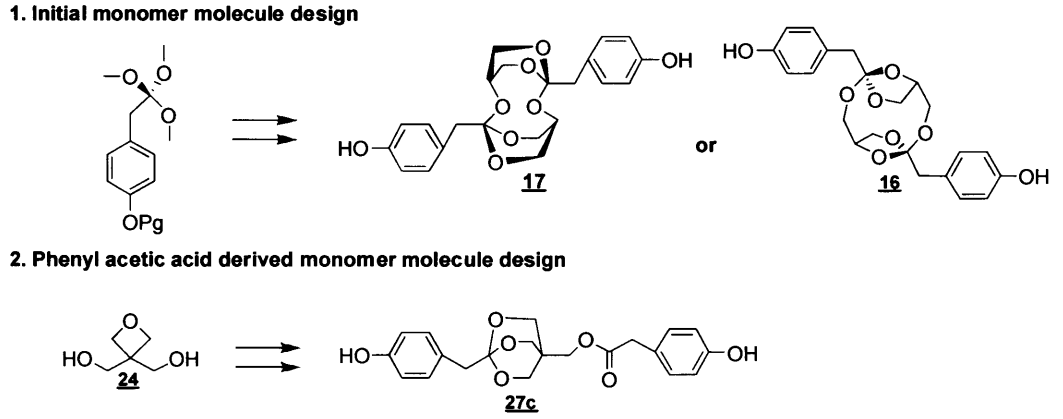



**Figure 24:** The structure property relationship between the initial monomer **17** and **16** with the phenyl acetic acid derived monomer **27c**.

The improved monomer **27c** has rationally evolved from the initial monomers **17** or **16** in both design and synthesis (Figure 24). Although different synthetic strategies were employed in both synthetic routes, they share similarities in molecular structure. These similarities have evolved to produce improved orthoester monomer **27c** in the following manner:

1. Improved orthoester stability within the monomer was observed with the bicylic [2.2.2] orthoester monomer **27c**. Furthermore, monomer **27c** has a

cyclic orthoester ring arrangement, while the initial monomers **16** and **17** have a acyclic orthoester ring arrangement within the structure as a whole (Figure 25). That is to say the oxygen bonded to carbon in forming the orthoester bond in initial monomer **16** and **17** is arranged in an acyclic ring. Which may have contributed to this types of orthoesters being less stable.

2. Phenyl acetic acid derived monomer design producing increased propensity for monomer to be potentially biocompatible.

3. Increased monomer yields due to an increase in hydrophobicity incorporation into the monomer design.

4. A limited number of four synthetic steps involved in the monomer synthesis.

5. Phenyl hydroxy polymerisible functionality incorporated into the monomer for potential *co*-polymerisation with commercially available monomers. Thus providing a route for producing libraries of poly(ortho esters) *co*-polymers.

6. One possible isomer preparation.

7. The bridged cyclic orthoester ring arrangement of molecule **27c** has increased the rigidity of the monomer. This rigid monomer characteristics can be transferred into the poly(ortho esters), producing materials with high modulus and strength. A requirement for processibility of degradable polymers.

8. Monomer **27c** has been found to be stable enough for ease of handling and storage.

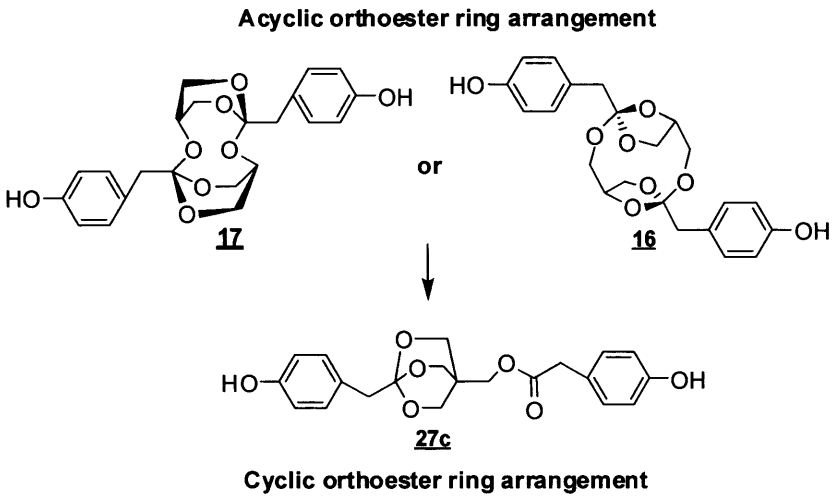



**Figure 25:** Structure property relationship between the initial monomer **17** and **16** with the phenyl acetic acid derived monomer **27c**, showing stability of different types of orthoesters.

The possible degradation profile in slightly acidic conditions of the phenyl acetic acid derived bicyclic [2.2.2] orthoester monomer **27c** is shown in Figure 26. The final degradation products of the monomer are the naturally occurring metabolite phenyl acetic acid and the water soluble pentaerythritol. In general the potential degradation profile occurs in two separate steps, firstly the bicyclic [2.2.2] orthoester ring protonates to facilitate a nucleophilic attack by water on the carbon to which all the three oxygen atoms are bonded to **28a**, this produces a cyclic acetal and terminal hydroxyl **28b** (Figure 26). Compound **28b** undergoes a further degradation to prepare the diester **28c** with terminal diol. The second step involves, diester **28c** (Figure 26) that becomes much more hydrophilic and allows water in and can potentially increase the rate of degradation. This is a trigger that can potentially be exploited, because if the monomer was used in a polymer then polymer morphology will change from being very hydrophobic to becoming hydrophilic. The ester of compound **28c** protonates and undergoes an attack by water to finally generate the much more hydrophilic triol ester **28d** and phenyl acetic acid **28e** (Figure 26). The triol ester **28d** then undergoes rapid degradation especially with the additional phenyl acetic acid generated as by-products which may catalyse the degradation further. The final step of the degradation processes yields the final products as being phenyl acetic acid **28e** and pentaerythritol **28f** (Figure 26).

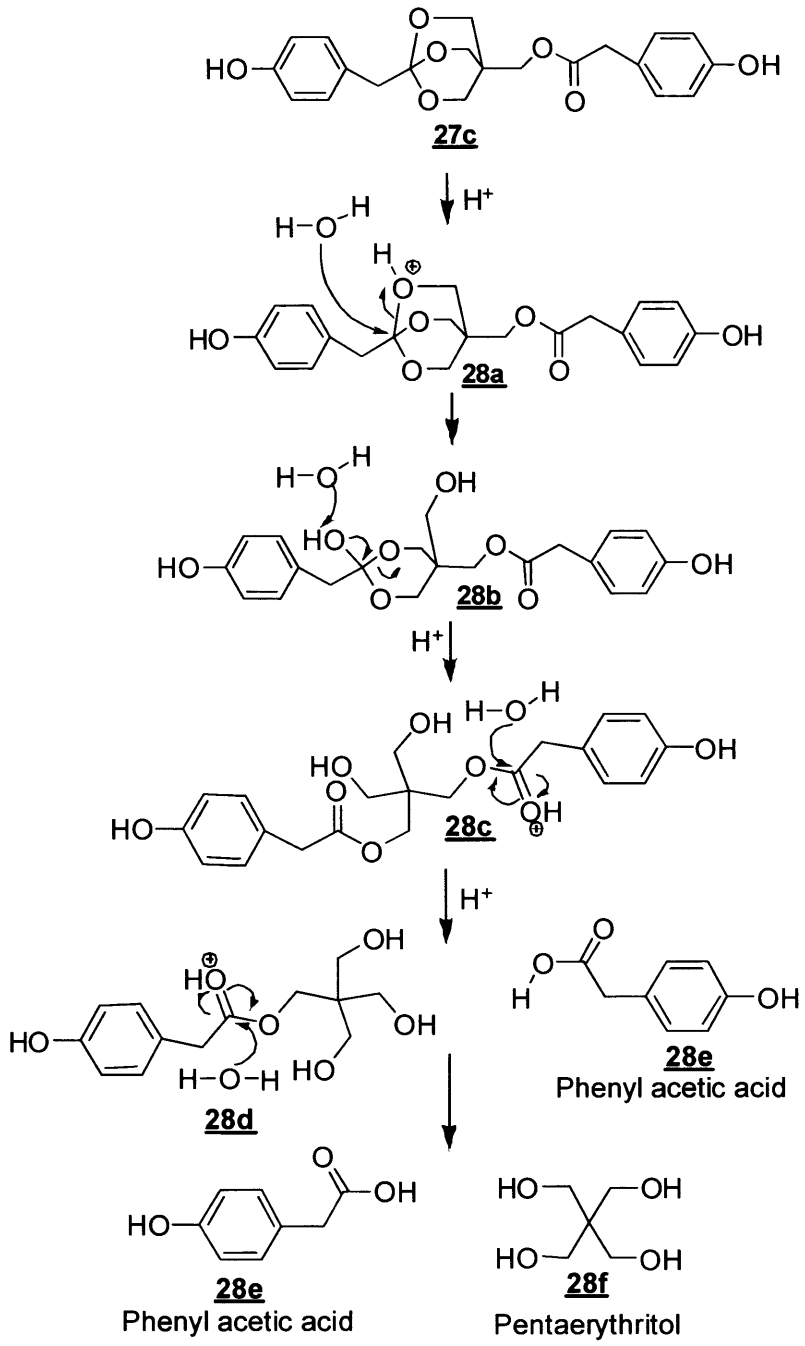



**Figure 26:** Possible orthoester monomer **27c** degradation profile producing the degradation products as phenyl acetic acid and pentaerythritol.

The orthoester monomer **27c** can be used to prepare a unique set(s) of polymers that can potentially undergo an erosion triggering hydrolysis. The hydrophobic properties incorporated into the monomer design were achieved by the use of phenyl acetic acid. The two phenyl groups of monomer in **27c** give hydrophobic capabilities, while

the orthoester bonds provide acid labile degradable capabilities (Figure 27). Furthermore, unique acid labile polymers can potentially be prepared by using the orthoester monomer **27c**. These acid labile polymers can used to exploit the erosion dependent triggering hydrolysis of the orthoester monomer **27c**.

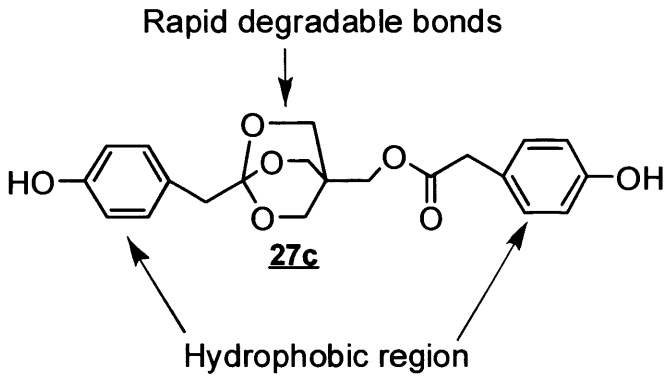



**Figure 27:** Potential surface erosion capabilities a polymer that is potentially prepared using the phenyl acetic acid derived orthoester monomer **27c**.

# Chapter 4

## *Design & synthesis of poly(ortho esters) co-polymers*

# Chapter 4

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# *Design and synthesis of poly(ortho esters) co-polymers*

## *4.1 Introduction: Traditional synthetic methods of preparing poly(ortho esters) co-polymers*

Generally, existing poly(ortho esters) are prepared by polymerisation reactions centred on the formation of the orthoester functionality during the polymerisation step. This approach has harboured some synthetic challenges in biomaterials research, because the sensitive orthoester bonds are formed in the polymerisations steps. The challenges and synthetic issues that need to be addressed include:

1. Existing poly(ortho esters) are predominantly prepared using acid catalysed polymerisation reactions. The acid catalyst of the reactions tends to degrade polymer and inhibit the polymerisation from being efficient.
2. Extremely dry polymerisation reaction conditions are required e.g. dry boxes to limit degradation of growing polymer chains.
3. Uncontrollable molecular weight.
4. Spontaneous de-polymerisation.
5. Scale-up reactions are costly due to expensive experimental requirements.
6. Most poly(ortho esters) that are prepared are thick oils or gel like materials.
7. Existing poly(ortho esters) do not address clinical need satisfactorily, as these materials do not have optimal processibility properties e.g. existing poly(ortho esters) are generally oil to gel like materials.

### *4.1.1 A new method of preparing poly(ortho esters) co-polymers.*

To address these challenges a new approach to poly(ortho esters) synthesis was needed. The new approach must incorporate the degradable orthoester functionality into the polymer other than that is formed during the polymerisation steps. One

possible solution is to produce degradable orthoester functionality into a monomer, as mentioned in chapter 3. The monomer **27c** (Scheme 18 (chapter 3)) will possess the polymeriseable functionality on either side with the degradable orthoester element embedded within the monomer, which is *co*-polymerised with commercially available monomers. Thus, a new generation of poly(ortho esters) *co*-polymers could be produced that attempt to addresses the synthetic and material limitations of traditional methods of preparing current poly(ortho esters) *co*-polymers (Figure 28). The new method relies on preparing acid labile degradable poly(ortho esters) in two separate stages.

1. Preparation of a stable orthoester monomer.

2. Polymerisation of the orthoester monomer with other monomers to prepare poly(ortho esters) *co*-polymers.

The basic aim of the new method of preparing poly(ortho esters) is to addresses the inefficiency of the polymerisation reactions used to prepare existing poly(ortho esters).

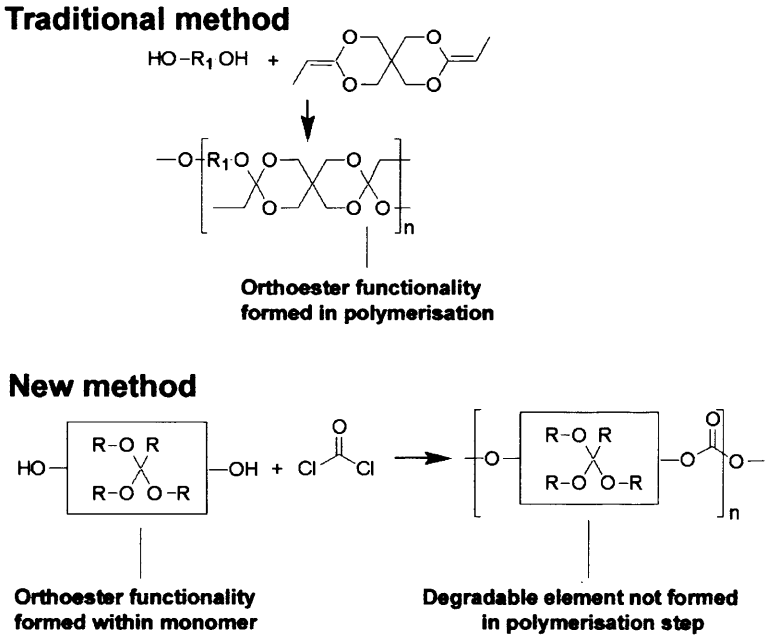



**Figure 28:** New approach to preparing a new examples of poly(ortho esters).

The advantages of preparing a new generation of POE co-polymers include:

1. Robust reaction conditions can be used in the polymerisation reaction, providing less chance for spontaneous depolymerisation, and a more manageable controlled molecular weights and mechanical properties.

2. POE *co*-polymers can be prepared that are rigid, and have high Tg values, providing a significant advantage over current poly(ortho esters) that have limited in biomedical applications, mainly due to gel to oil like physical properties. We seek to address these issues by preparing a solid, and processible poly(ortho esters).

3. The new method has the potential commercial advantage of being cost effective, particularly in the scale-up reactions, where expensive experimental equipments are not required.

4. The new method of preparing poly(ortho esters) opens up pathways to preparing tailor made polymers. As the orthoester monomer **27c** (Scheme 18) described in chapter 3 can be *co*-polymerised with commercially available monomer, providing a means of preparing a 'small tailor' made polymer library. The potential tailor made polymer library has a better chance to better match: the clinical, mechanical, processing and fabrication requirements of polymer biomaterials, which the four existing poly(ortho esters) do not satisfy, as prepared by Heller et al[35;36;46;66]. Tailor made specific biomaterials that are used to address specific clinical problems has been a major challenge in the biomaterial and pharmaceutical formulation fields

### *4.1.1 Tailor made biomaterials*

The four existing poly(ortho esters) have limited clinical application, because the four families of biomaterials have a gel to oil like physical properties, which was mainly developed by Heller et al[36]. In certain biomedical applications these properties have been utilised in a specific pharmaceutical formulation research and

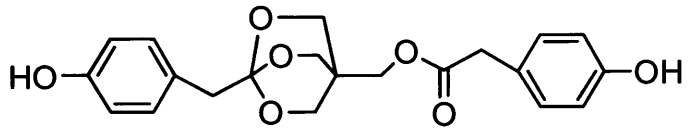
applications, where the drug incorporation into the polymer matrix is done by room temperature mixing[106]. This is a significant advantage and currently being commercialised by AP Pharma in drug delivery applications[59]. There is a clinical need for biomaterials that are tailor made to better match the application specification, both in design and performance.

The new method of producing poly(ortho esters) that can be tailor made for specific biomedical applications, and can potentially open doors for biomaterial research other then the pharmaceutical formulation research[36;66]. We have proposed to prepare the tailor made biomaterials that better match the clinical applications need, by preparing the poly(ortho esters) using the new method of preparing poly(ortho esters) (Figure 28). Using the orthoester embedded monomer method (new method) a POE or a series of Poly(ortho esters) can potentially be prepared by changing the commercially available monomers used to form the polymer (Figure 28). This produces opportunities to prepare biomaterials that can have incremental changes in physical and mechanical properties, which can be used to identify and select the required biomaterial from the polymer library. These incremental changes in material properties include the possibility of preparing poly(ortho esters) as biomaterials that posses a relatively high modulus and strength. This has been a major draw back of existing poly(ortho esters). In addition, we hoped this new method would further address the synthetic and material properties limitation that we came across in the polyacetals work (chapter 2), using the traditional method of preparing acid labile polymers. The preparation of biomaterials particularly acid labile polymers that satisfies the required clinical need has been a major challenge in the biomaterials field e.g. drug delivery[37]. Mainly a few handful of the acid labile polymers are often routinly used with adjusting ('fine tuning') these materials properties to better match the clinical need, an example being the polyesters poly (glycolic-lactic acid (PLGA)). These PLGA are used from drug delivery to medical implant application etc. There has been a major lack of new materials available that have been precisely designed and synthesised for the specific clinical problems and needs.

### *4.1.2 Phenyl acetic acid derived bicyclic [2.2.2] orthoester monomer used to prepare a new generation of poly(ortho esters)*

The symmetrical bicyclic [2.2.2] orthoester monomer molecule derived from phenyl acetic acid **27c** (Scheme 18) will be used to prepare the new poly(ortho esters). The bicyclic orthoester [2.2.2] ring provides a rigid monomer. It is expected that the rigid properties of the monomer will impart its properties to the polymer when it is co-polymerised with commercially available monomers. In addition, to the polymer biomaterial exhibiting the high modulus and strength, the poly(ortho esters) have the potential to undergo degradation by an surface erosion triggering hydrolysis mechanism. These acid labile polymers can used to exploit the erosion dependent triggering hydrolysis of the orthoester monomer **27c** (Scheme 18) based *co*-polymers.

This provides an opportunity to prepare a material that maintains its mechanical properties throughout a significant portion of the degradation process (e.g. current acid labile bulk eroding polymers do not satisfy this criteria)[34].




**27c**

**Figure 29:** Bicyclic [2.2.2] orthoester monomer **27c**.

If monomer **27c** were *co*-polymerised with commercially available non-toxic monomers, then tailor made poly(ortho esters) co-polymers could be prepared (Figure 29).

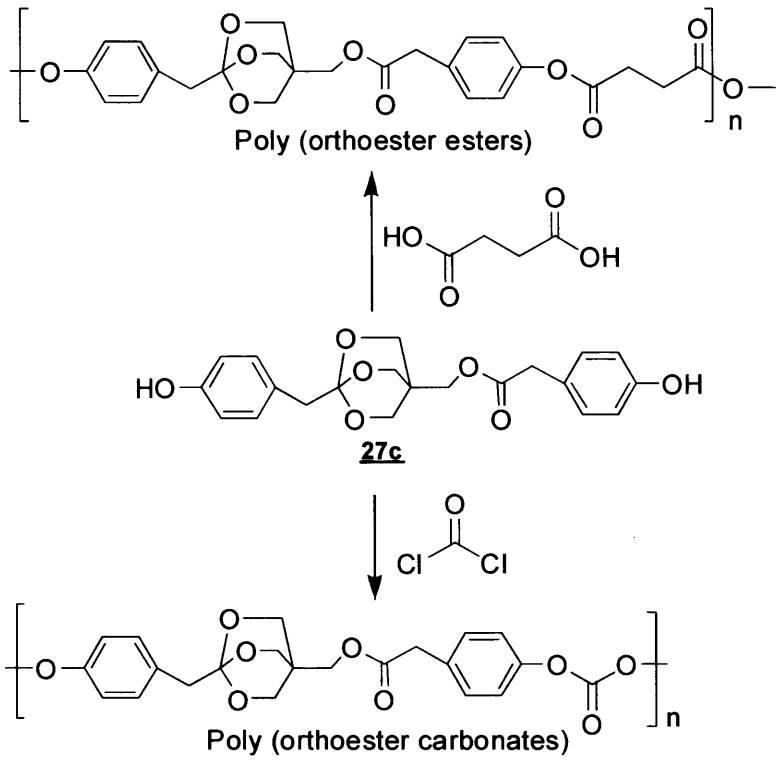



**Figure 29a:** Examples of tailor made co-polymers that can potentially be prepared using the orthoester monomer 27c.

## *4.2 Results and Discussions*

### *4.2.1 Poly (orthoester carbonates) derived from 4-Hydroxy (phenyl acetic) acid bicyclic [2.2.2] orthoesters monomer (27c)*

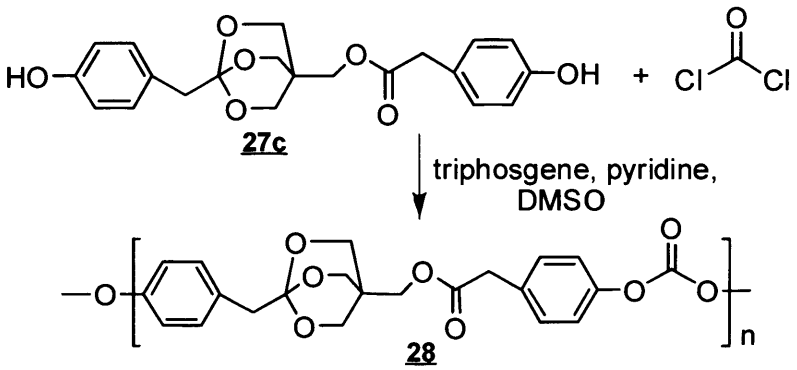
The first polymerisation to be attempted with monomer **27c** (Figure 29) was to examine copolymerisation with phosgene surrogates to give polycarbonates. When polymerising the bicyclic [2.2.2] orthoester monomer **27c** to produce a new POE, carbonates were incorporated into the polymer design. Because the incorporation of carbonates into the polymeric chain has the advantage of producing a material that has processible and compliant material characteristics. Polycarbonates are

recognised for their processible advantages due to the rigidity of the carbonate bonds[74;107]. The incorporation of the carbonate bonds into the polymer mainchain of POE **28** (Scheme 18) has the following desirable features:

1. Better mechanical and processible properties.
2. A solid material.

The orthoester monomer embedded design used to prepare a new POE *co*-polymer (Scheme 19), is a first example of the preparation of a highly degradable co-polymer that potentially possesses high Tg values and processible properties.

Commercially available triphosgene was used to generate the reactive species phosgene in-situ using the base pyridine in anhydrous toluene. This solution was added drop-wise at 0 °C to a solution of monomer **27c** dissolved in anhydrous DMSO. The polymerisation was stirred at 0 °C under a flow of argon. After one hour, the polymerisation solution started to get viscous. The polymerisation was left at room temperature overnight, then dissolved in $CH_2Cl_2$ and washed with aqueous ammonium chloride solution. The solution was dried over sodium sulphate and concentrated. The polymer was isolated by precipitation from water to give the poly (orthoester carbonates) **28** (Scheme 19) MW (7,000 g / $mol^{-1}$) PDI (2.3) [107]. A series of solvents (water, methanol, hexane and ether) was used to precipitate the polymer, which resulted in the best precipitation in water (containing a few grains of sodium bicarbonate to limited potential degradation) producing a mass recovery of 23 % (w/w).




**Scheme 19:** Poly (orthoesters carbonates) **28** synthesis derived from bicyclic [2.2.2] orthoester monomer **27c**.

Poly (orthoester carbonates) **28** had oil like physical properties due to the low molecular weight of the polymer. It was assumed the low molecular weight may have attributed to the non-competing anhydrous reaction conditions, where the reaction solvent DMSO may have some adventitious water present, and therefore causing polymeric mainchain breakage. The small amount of adventitious water present may have reacted with phosgene, reducing the propensity of reaching a 1:1 stoichiometric ratio between the orthoester monomer **27c** and the phosgene, producing unreacted groups at the polymer chain ends.

Attempts were made to use THF as the reaction solvent as it was less hygroscopic and it was hoped this would ensure experimentally feasible anhydrous conditions and further improve the propensity for preparing a high molecular weight polymer. The polymerisation yielded poly (orthoester carbonates) **28** MW (12,000 g / $mol^{-1}$) PDI (2.6) as a semi oil like material. Although the molecular weight of the polymer **28** was slightly higher, the synthesis was not taken any further due to the toxic nature of working with phosgene.

An alternative method to prepare these *co*-polymers was examined using *p*-chloronitrophenol as the polymeric chain formation was investigated (Figure 30). The study prepared the POE *co*-polymer **28** as an oily material and the molecular weights were not higher then the phosgene method previously investigated (Scheme 19)

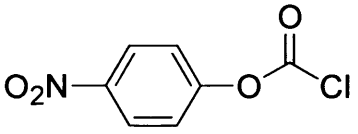



**Figure 30:** *p*-Chloronitrophenol as a coupling agent in the preparation of poly(ortho ester) *co*-polymer **28**.

The study has revealed that the orthoester embedded monomer was stable in the set of polymerisation reaction conditions used. A small molecule acid catalyst was not required in the polymerisation reactions to produce the poly(ortho esters). The bicyclic [2.2.2] orthoester embedded monomer polymerisation reactions had the advantage of no orthoester bond degradation occurring. The investigation has shown

for the first time that a degradable acid labile polymer can be prepared without the use of an acid catalyst, or other small molecules present that degrades the acid liable bonds traditionally formed in the polymerisations. This is routinely used to prepare current poly(ortho esters) where the acid catalyst degrades the growing polymeric chain. We have also shown that no spontaneous depolymerisation has occurred, which is often found in the traditional method of preparing poly(ortho esters)[46].

#### *4.2.1.1 Poly(ortho esters) prepared by step-growth polymerisation*

The co-polymer **28** (Scheme 19) preparations involved a step-growth polymerisation. Generally, existing poly(ortho esters) and other acid labile biodegradable co-polymers are also prepared using step-growth polymerisation [35;36;65]. Achieving high molecular weight of co-polymers using step-growth polymerisation has many challenges, especially when synthesising degradable acid labile polymers, an acid catalyst is generally involved. Step-wise polymerisation occurs by consecutive reactions in which the degree of polymerisation and the average molecular weight of the products increase as the reaction proceeds. These types of reaction are generally recognised to be reversible, therefore the eliminated by-products must be removed from the polymerisation system to achieve high molecular weight products. Step-growth polymerisation involves the building of small blocks of dimers, trimers, tetramers etc until the end of the polymerisation where high molecular weights are achieved (Figure 31).

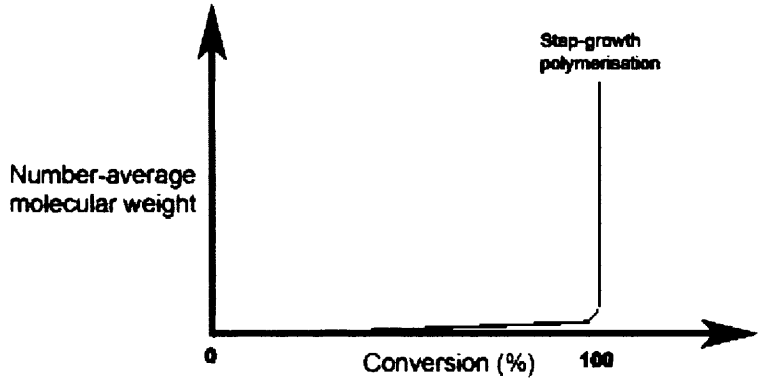



**Figure 31:** Number average molecular weight of step-growth polymerisation, showing high molecular weight achievement at high conversion rate.

In achieving this high molecular weight the polymerisation reaction system must have the stiochiometric ratio of the monomers involved as close to a 1:1 ratio. This enables reactive functional groups to be equal during the polymerisation. Also the purity of the monomer is essential in achieving high molecular weight co-polymers.

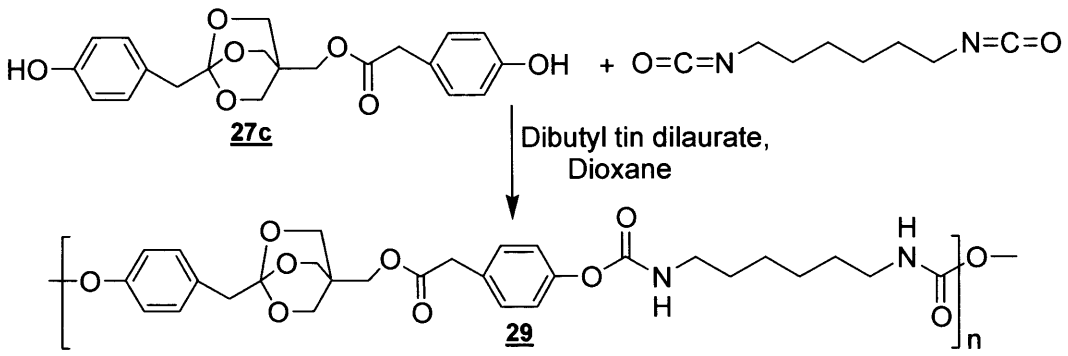
The low molecular weight of the poly(ortho esters) co-polymers **28** (Scheme 19) that was prepared may have been as a result of not achieving a 1:1 monomer ratio. Also the purity of the bicyclic [2.2.2] orthoester monomer **27c** (Scheme 19) may not have been analytically pure. This would be expected for a highly reactive reagent. Both of these factors may have contributed towards an inefficient polymerisation. To address these issues the bicyclic [2.2.2] orthoester monomer **27c** must be further purified using recrystallisation techniques. The stiochiometric ratio of monomer **27c** and the reacting monomer needs to be as close to a 1:1 ratio as possible.

### 4.2.3 *Poly (orthoester urethanes) 29 derived from 4-hydroxy (phenyl acetic) acid bicyclic [2.2.2] orthoesters monomer 27c*

The first polymerisation to be attempted with monomer **27c** (Figure 29) was to examine copolymerisation with urethanes to give polyurethanes. Polyurethanes have similar mechanical properties as polycarbonates that were previously attempted, particularly with co-polymers **28** (Scheme 19). We attempted commercially available reactive urethane monomer and copolymerised with orthoester monomer **27c** to produce the poly(ortho esters) **29** (Scheme 20).

The formation of urethane co-polymers requires less hazardous and toxic experimental conditions. Urethanes are widely recognised to possess excellent processible characteristics similar to polycarbonate co-polymers, due to the urethane bonds having mechanical properties in-between an amide and an ester bond[74]. Urethane co-polymers derived from 1,4 -butane diisocyanate are recognised as biocompatible, as the diisocyanates degrade to biocompatible 1-4 butane diamine [108]. The bicyclic [2.2.2] orthoester monomer **27** (Scheme 19) was polymerised with commercially available 1,4-butane diisocyanate in the presence of a catalyst (Fe(III) acetyl acetone) to prepare the *co*-poly (orthoester urethane) **29** (Scheme 20) as a

brown solid powder (22,000 g / $mol^{-1}$) PDI (3.1). The MW of these urethane *co*-polymers have improved compared to co-polymers **28** (Scheme 19).




**Scheme 20:** Poly (orthoester urethane) **29** prepared by a polymerisation of an orthoester monomer **27c** and a diisocyanate monomer.

*Table 5*: Catalyst effect on the polymerisation optimization for the preparation of poly (orthoester urethane) **29**.

| Entry[a] | Catalysts (molar %(w/w)[b] | MW (g $mol^{-1}$ )[c] | PDI |
|---|---|---|---|
| 1 | Fe (III) acetylacetone (1 %) | - | - |
| 2 | Fe (III) acetylacetone (3 %) | 13000 | 2.5 |
| 3 | Fe (III) acetylacetone (5 %) | 22000 | 3.1 |
| 4 | Fe (III) acetylacetone (10 %) | 14000 | 2.2 |
| 5 | Fe (III) acetylacetone (20 %) | 9000 | 1.9 |
| 6 | Dibutyl tin dilaurate (5 %) | 3000 | 3.8 |
| 7 | Dibutyl tin dilaurate (10 %) | 1500 | 4.6 |

[a] Experimental procedures (A) are described in the Experimental Section. [b] 100 mg equivalent monomer ratio in DMSO (2 ml) at room temperature and stirred overnight. [c] Conventional GPC calculation against polystyrene standards in THF solvent system.

This investigation produced an acid labile poly(ortho esters) co-polymer **29**, without the use of harsh anhydrous reaction conditions. We have shown a way of preparing a first generation of poly(ortho esters) *co*-polymers, where the degradable elements are not formed during the polymerisation reactions. In conclusion, as for poly(ortho esters), there is a need for better synthetic methods to prepare these

polymers more easily without the need of stringent anhydrous conditions, with more broad structural variation, and in a more cost effective manner. We have shown examples of poly(ortho esters) preparation, the synthesis involved the use of a novel stable orthoester monomer. The key aspect was to embed the orthoester within the monomer while provide orthogonal polymerisation functionality. This orthogonal polymerisation functionality of the orthoester monomer was used in co-polymerisation with commercially available monomer(s), to prepare novel poly(ortho esters) *co*-polymers. This synthetic route attempted to show a way of addressing the synthetic limitations for the preparation of existing poly(ortho esters), and it is believed to be the first such example.

# Chapter 5

## *Alternative synthetic routes*

# Chapter 5

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## *Alternative synthetic routes*

### *1.0 Introduction: orthoester monomer design and specifications*

Several synthetic approaches were examined to prepare acid labile poly(ortho esters) that are described in Chapter 4. The basic hypothesis throughout had been to embed the degradable element into a stable monomer. This chapter details the orthoester monomer synthetic routes and poly(ortho esters) co-polymer(s) synthesis that were investigated early on at the beginning of the research.

The research presented within this chapter aims to show, the synthetic routes that we examined for information gathering or just had to be given up due to experimentally not feasible. A better understanding of orthoester monomer specifications and requirements is needed. In some instances the information gathered from the synthesis was used and reincorporated into the monomer synthesis design and to prepare a better synthetic route. It was hoped this would enable a better chance to addressing our hypothesis as outlined in chapter 1 (introduction section (aims)), which is to prepare a stable orthoester monomer.

Several routes to monomers were examined, which resulted in in-efficient synthesis, oily monomers or there was the difficulty of purifying the monomers. Therefore, some synthetic routes were abandoned due to the few reasons as mentioned above. However, we have shown different ways of preparing new orthoesters monomers, which can potentially be exploited by other research groups.

The first part of the chapter is concerned with preparing a new generation of poly(ortho esters) *co*-polymers. This is prepared with the orthoester degradable element embedded within a monomer and this is co-polymerised with commercially

available monomers to prepare poly(ortho esters) (Route 1 (Figure 32)).

The second part of this chapter is concerned with the preparation of poly(ortho esters) *co*-polymer(s) from precursor *co*-polymers (Route 2 (Figure 32)). We have shown here a new way of preparing poly(ortho esters) *co*-polymers from precursor polymers using a pH triggered rearrangement reaction technique (stimuli responsive polymers). Stimuli responsive polymers alter their physicochemical properties as a result of external environmental changes[109]. The environmental trigger (changes) behind these physicochemical property transitions can come from a external stimuli such as a change in temperature or as we will shown in our case a change in pH, or in some cases a change in ionic strength[110]. A typical pH-sensitive polymer, undergoes protonation/deprotonation events occur which impart the charge over the molecule (generally on the carboxyl or the amino groups), therefore it depends strongly on the pH. The pH-induced phase transition of pH-sensitive polymers tends to be very sharp and usually switches within 0.2 – 0.3 unit of pH[111]. Examples of stimuli responsive polymers that change their physicochemical properties at low pH include co-polymers of methylmethacrylate and methacrylic acid. These polymers undergoes a sharp conformational transition and collapse at a low-pH of around 5[112].

We have examined and prepared new poly(ortho esters) *co*-polymers that are prepared using the pH triggered rearrangement reaction on a precursor polymer method for the first time. The advantages of using this new way of preparing poly(ortho esters) *co*-polymers are highlighted, as well as the problematic issues associated with using this type of polymerisation techniques that we have reported. The first stage of poly(ortho esters) preparation using the pH triggered rearrangement reaction involves a precursor co-polymer synthesis. The second stage involves stimuli triggered reactions that facilitate an intra-molecular rearrangement reaction yielding the desired poly(ortho esters).

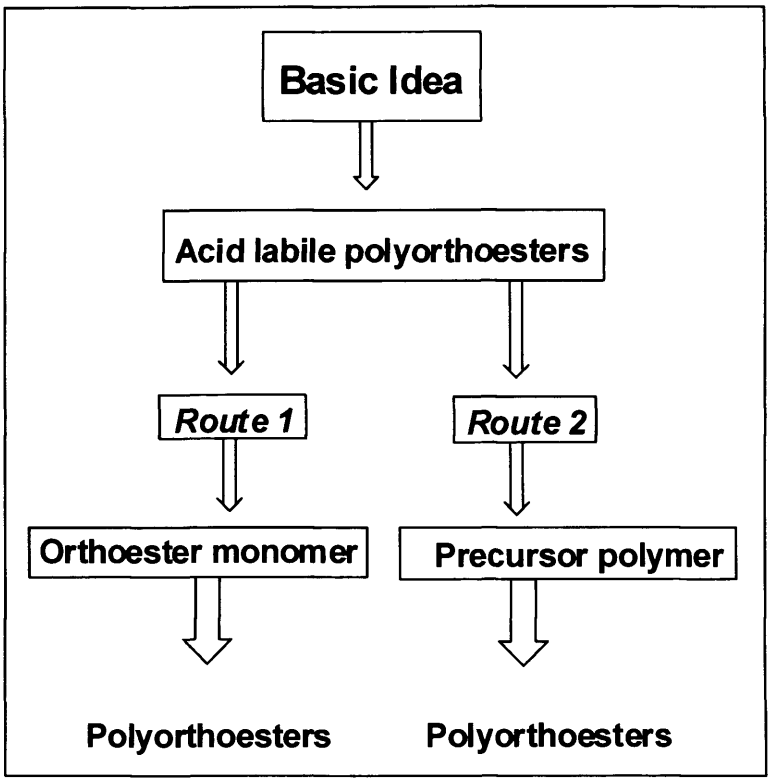



**Figure 32:** Schematic illustration of poly (ortho esters) synthetic strategy.

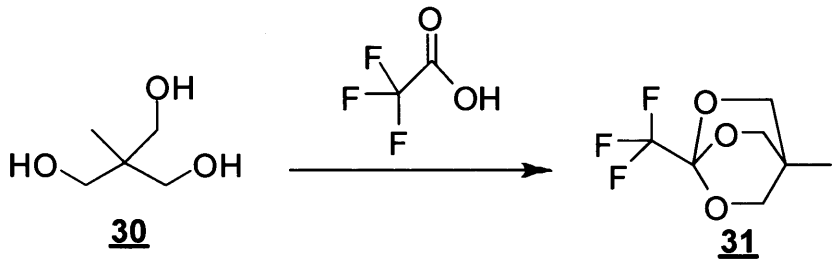
The synthetic routes that are reported herein were stopped or the resulting products did not satisfy the monomer specifications that were outlined in chapter 3. This was predominately due to in-efficient synthesis, oily monomers or there was the difficulty of purifying the monomers. However, in some cases within this chapter the aims were to get an early idea of the challenges and monomer properties that needed fine tuning before the monomer and polymer synthesis can be achieved successfully. In addition, the second aim of this chapter was to test the idea of preparing a new generation of poly (ortho esters) using pH triggered rearrangement reaction. We believe pH triggered rearrangement reaction, particularly for poly(ortho esters) may open doors to preparing highly degradable co-polymers as novel biomaterials or biomedical polymers. This polymerisation technique may provide a more manageable and controllable synthesis. Also the vulnerable phase of preparing high molecular weight co-polymers is limited, as the polymer molecular weight is incorporated within the precursor polymer, and not in the final polymer.

# 2.0 *Results and Discussion*

## 2.1 *Introduction: Bicyclic [2.2.2] orthoesters*

### 2.1.1 *Model bicyclic [2.2.2] orthoester derived from 2-hydroxymethyl-2-methyl-1, 3-propanediols*

The model orthoester monomer synthesis was studied to get a better understanding of a bicyclic [2.2.2] orthoester and in particular, the stability characteristics of these types of orthoesters and the ease of handling of these orthoesters in the laboratory and storage of the compounds. The synthesis involved a reaction of a triol **30** with a strong carboxylic acid to produce the bicyclic orthoester **31** (Scheme 21). We pursued to prepare different but related or semi-related synthetic orthoester monomer pathways to assess the types of synthetic routes that would yield a successful orthoester monomer. More particularly we examined the widely studied bicyclic [2.2.2] orthoesters e.g. **31** (Scheme 21), as a protecting group for carboxylic acid in small molecule synthesis. We believed through this approach we may possibly be able to identify a set of characteristics or reactions conditions that could be used to prepare an orthoester monomer with relative ease.

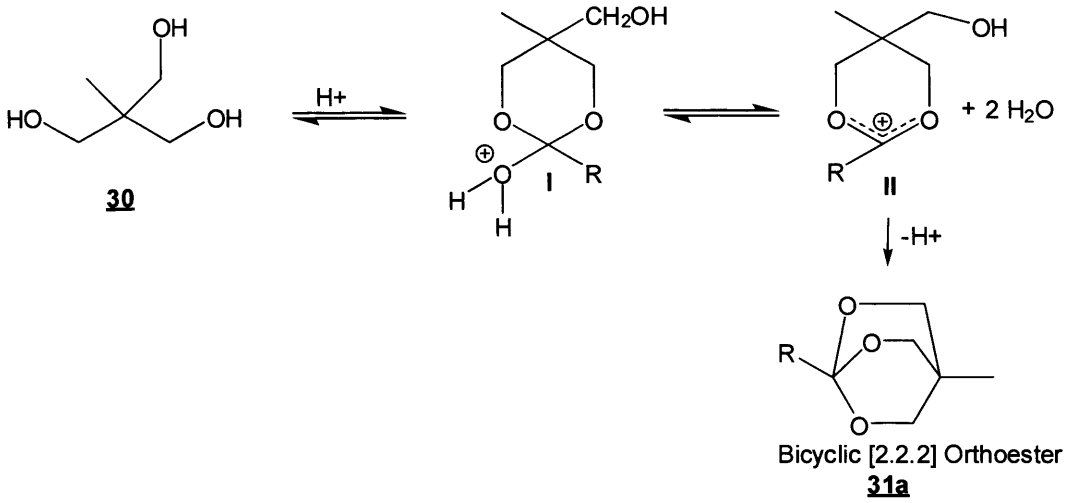



**Scheme 21:** Model bicyclic [2.2.2] orthoester **31**.

Barnes et al successfully prepared bicyclic [2.2.2] phosphates using 2-hydroxymethyl-2-alkyl-1, 3-propanediols[113]. The stability and the success of the bicyclic [2.2.2] bridged ring arrangement led the investigators to apply this theory

using other elements e.g. carbon. As the carbon series were investigated, a bicyclic [2.2.2] ring arrangement based on the carbon series proved to be relatively stable. Furthermore, the favourable stability characteristics of the carbon ring arrangement and the synthetic route used for product preparation was practical and did not harbour any difficult synthetic conditions or requirements.

Barnes et al realised that by choosing a solvent which had a boiling point higher then water, but less then 150°C (triol **30** decomposed) pushed the equilibrium forward[113]. The strength of the acid also affected the yield of the reaction. That is to say the greater the electro-negativity of the substituent on the acid group, the greater the contribution it has in moving the reaction equilibrium to product formation. Barnes et al have suggested that the intermediate **II** (Scheme 22), with the positive charge distributed over the two oxygens and carbon, may have been stable enough to react with water to regenerate **I**. However, the authors noticed that by having an electron attracting R group it could inductively increase the positive charge on the carbon atom of **II**, hence facilitating an attack by a base, in particular by the third hydroxyl group yielding an orthoester.




**Scheme 22:** Bicyclic orthoester **31a** ring arrangement from a carbon series[113].

An example of a Barnes et al method being applied included the reaction of benzotrichloride, 2-hydroxymethyl-2-methyl-1, 3-propanediols **30** and a base (triethylamine)[113]. The reaction produced the undesired tetrachlorodibenzyl, even upon promoting $S_N1$ reactions[113]. The authors attempted a direct esterification of the triol **30** with an acid based on the rationale that the same factors that makes preparation of ketal from ethylene glycol successful, whereas, simple alcohol fail can be applied here.

We used Barnes et al suggestion and designed the reaction of a direct esterification of trichloroacetic acid and the triol **30** to produce the bicyclic orthoester monomer **31** (Scheme 21), which has not been previously reported. The preparation of the bicyclic [2.2.2] orthoester **31** (Scheme 21) was identified using $^1$H-NMR (see experiment section, chapter 6). We attempted the synthesis and came across difficulties in respect of the stability of the molecule. This was mainly in the separation and isolation of the crude product from the impurities which were possibly by-products of the reaction. To address this issue of product stability, the crude acid labile products **31** were separated under basic conditions when performing the chromatography. The compound **31** was purified by chromatography on silica with the mobile phase containing small amounts of triethylamine, to keep the system

basic enough to inhibit the acidic silica (a Lewis acid) from hydrolysing the orthoester bonds.

The difficulty with the stability of these types of orthoester ring arrangements led us to consider other orthoester ring arrangements, which can be used to prepare a successful orthoester monomer, which satisfies the monomer specification as outlined in chapter 3 e.g. monomer stability. To address associated problems with bicyclic [2.2.2] orthoester **31** and **31a** (Scheme 21 and 22), we searched the literature to find examples of other types of orthoesters, where orthoester functionality arrangement is arranged in such a way that it makes these types of orthoesters stable. The literature search produced ABO-type orthoester molecules, also known as bicyclic [3.2.1] orthoesters as shown in Figure 33.

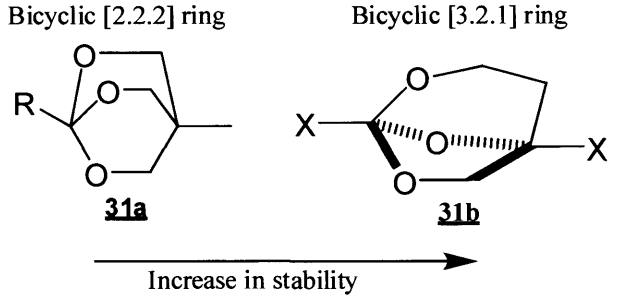



**Figure 33:** Stability comparison of bicyclic orthoester **31a** and **31b**.

There are a number research groups that have been researching bicyclic [3.2.1] orthoester as a protecting group used in synthesis of small molecules, of which Wipf et al group have been most active to date[81;114]. Although these types of orthoesters have been widely reported, there are no examples of a bi-functional monomer structurally incorporating the bicyclic [3.2.1] orthoester ring arrangement (Figure 33).

### *2.1.2 Zirconocene catalysed bicyclic [3.2.1] orthoester from epoxy esters*

Wipf et al have studied the use of cationic zirconocene species prepared in situ from organozirconocene and catalytic amounts of $AgClO_4$. This was used to facilitate

initiating a tandem epoxide rearrangement-aldehyde addition cascade reaction. The reaction produced a range of dioxolanes **31c** and orthoesters **31d** (Figure 34). From this research it became evident that the carbon chain length alpha to the ester group determined the formation of a bicyclic orthoester or a dioxolanes (Figure 34). For example if the carbon chain length is one carbon length then a dioxolanes **31c** is prepared, while if the carbon chain length is two carbons in length then a bicyclic [3.2.1] orthoester **31d** is prepared (Figure 34). It has been suggested that mechanistically these bicyclic [3.2.1] bridge orthoester formations are related to Corey's $BF_3$-catalysed rearrangement of an acyloxetane to the 2.6.7-trioxabicyclo [2.2.2] octane (Bicyclic [2.2.2] orthoester )[98;98;115].

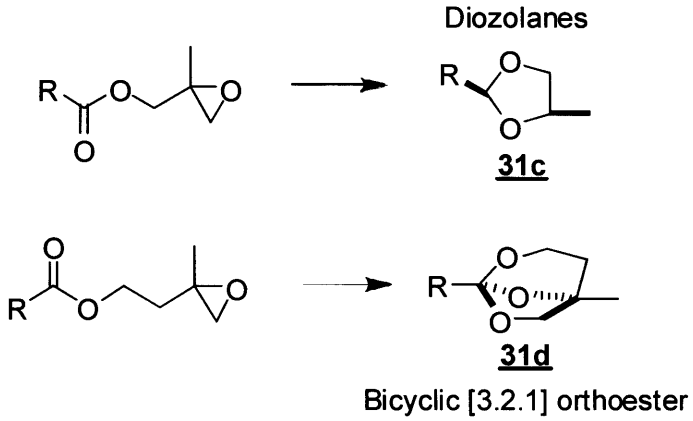
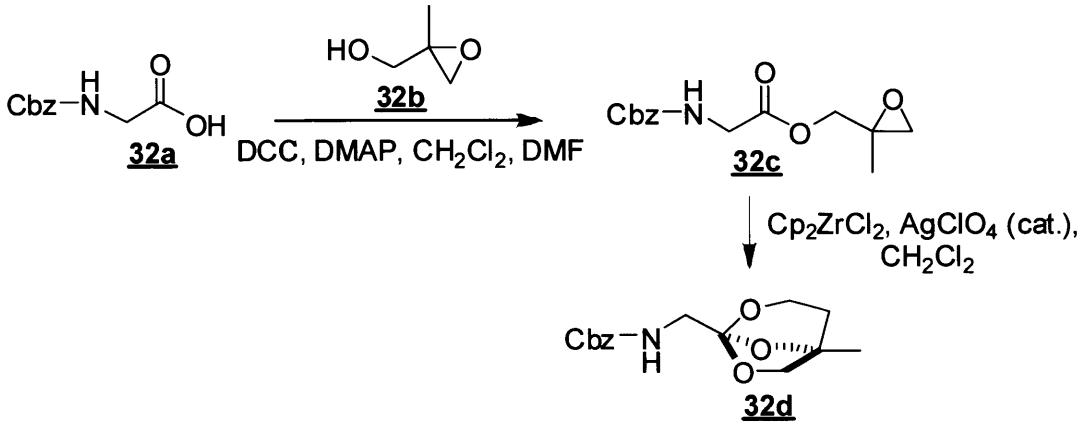



**Figure 34:** The effect of epoxy carbon chain length on formation of bicyclic [3.2.1] orthoester **31d** [91].

The mild conditions of the zirconocene catalysed reaction have provided a useful route for the formation of a bridge orthoester of polyfunctionalised carboxylic acids, in particular N-protected amino acids. Glycine **32a** (Scheme 23) for example as according to Wipf et al, undergoes a reaction with epoxy alcohol **32b** in the presence of dicyclohexycarbodiimide (DCC) and dimethylaminopyridine (DMAP), followed

by successive treatment with 10% of $CP_2ZrCl_2$ and 2 mol% of $AgClO_4$[114]. This led to the preparation of the amino acid orthoester **32d** (Scheme 23). The orthoester formation produced a protection against nucleophillic attack by hydroxide or organometallic reagents. The conversion of the carboxylate to orthoester provided a means of controlling the acidity of α –hydrogen.




**Scheme 23:** Glycine derived bicyclic [3.2.1] **32d** orthoester as prepared by Wipf et al[114].

### *2.2.0 Bicyclic [3.2.1] orthoester ring arrangement and their stability*

When it comes to the stability of these types of orthoesters, Wipf et al has shown that the bicyclic [3.2.1] orthoesters were far more stable compared to the Corey's bicyclic [2.2.2] orthoesters[98]. It was also suggested this may have been due to a significant difference in the Bronsted acid versus the Lewis acid liability of bicyclic [2.2.2] orthoesters **32e** and bicyclic [3.2.1] orthoesters **32f** (Figure 35). As the bicyclic [3.2.1] orthoesters are known to be far more stable then the bicyclic [2.2.2] orthoester ring arrangement. We believe this increase in stability would address the synthetic difficulty encountered in our earlier work related to the 2-hydroxymethyl-2-methyl-1, and 3-propanediols derived model bicyclic [2.2.2] orthoester (Section 2.1.1). For this reason, the bicyclic [3.2.1] orthoesters were studied to assess the likelihood of better matching the monomer specification as outlined in chapter 1 (sections: aims) and chapter 3 (section 2.3.5; rational evolution of monomer synthesis).

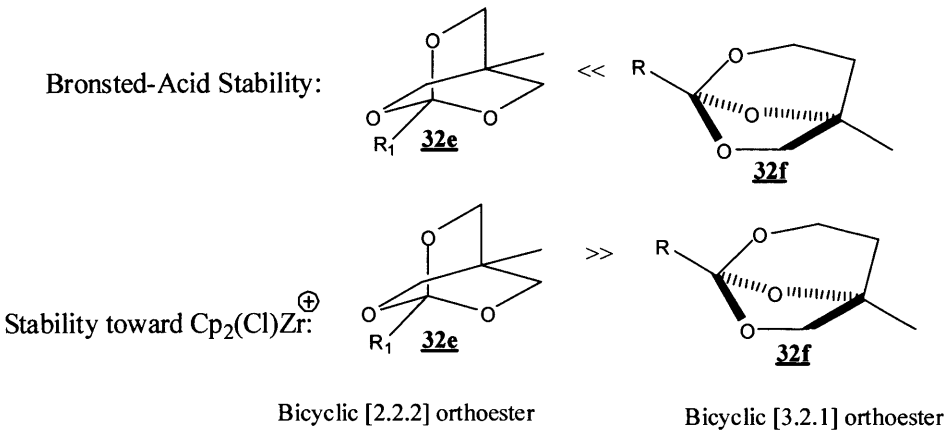
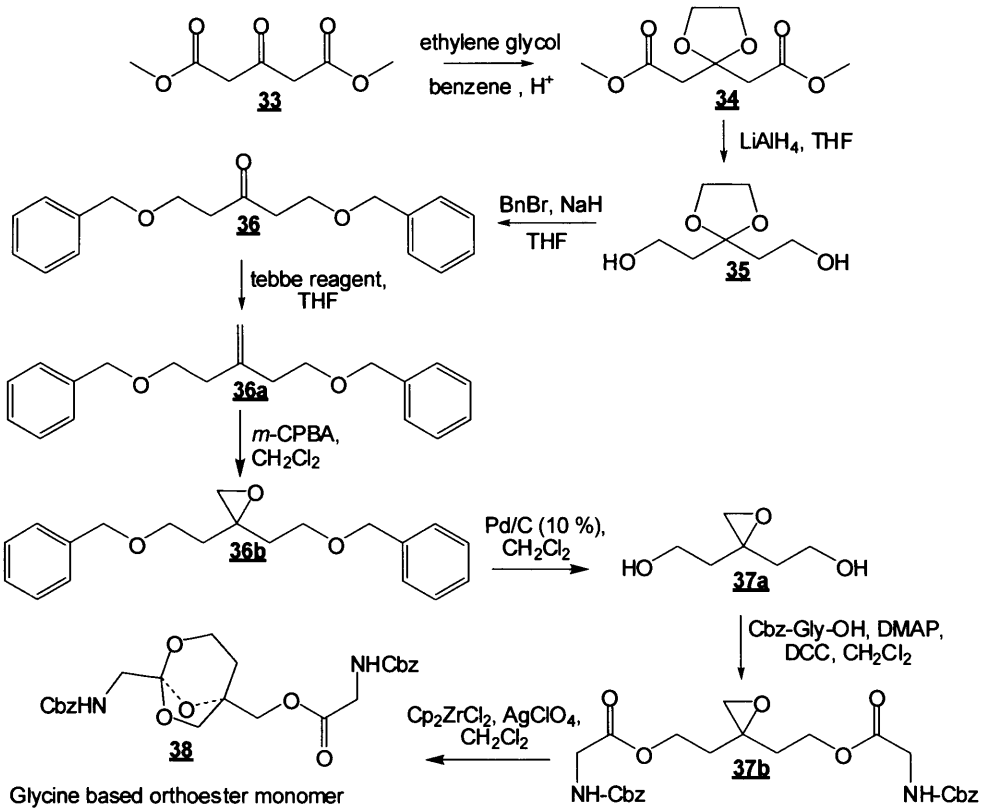



**Figure 35:** Bicyclic [2.2.2] orthoester **32e** and bicyclic [3.2.1] **32f** orthoester stability comparison towards a Lewis and a Bronsted acid [91].

### *2.2.1 Bicyclic [3.2.1] orthoester derived from amino acid epoxy ester*

The synthetic route for the synthesis of bicyclic [3.2.1] orthoester based monomer that we followed is shown in Scheme 24. These monomers were designed to be derived from naturally occurring amino acids in particular, glycine with the hope of preparing biomaterials that have a better chance of being bioresorbable. Dimethyl acetonedicarboxylate **33** (Scheme 24) was used as a starting material, because the compound already contains a five-carbon chain with the required 1,3,5-oxygenation pattern to reach the epoxy diol **35** (Scheme 24). Furthermore, dimethyl acetonedicarboxylate **33** is an inexpensive material, as well as being economically favourable if the reaction were to be scaled up. Text book protection group chemistry conditions were used to protect the ketone group of **33**, as the ester groups were reduced[60]. The ketone group was protected by an acid catalysed reaction using ethylene glycol to produce the dioxane **34** (67 %).




**Scheme 24:** Glycine derived bicyclic [3.2.1] orthoester monomer **38** synthesis based dimethyl acetonedicarboxylate **33**.

The reduction of **34** to **35** (Scheme 24) was achieved using $LiAlH_4$, the reaction conditions were used as described by Davenport *et al*[116]. Initially there were a few difficulties with the separation and isolation of the product. Particularly, when standard isolation techniques produced a low product yield recovery, mainly due to the high polarity of the molecule. However, this was addressed using Fieser's work-up to produce a product recovery yield of 79 %[61;61]. The low product recovery behaviour was further experienced in the deprotection step of the ketal **35**, where 1M aqueous HCl, acetone and THF provided a quantitative yield when done in small scale, but when scaled up a reduced yield was observed. This showed that the hydrophobic **36** (Scheme 24) was far more water soluble than expected. Therefore to

counterbalance this, a ten fold quantity reduction of 1M aqueous HCl provided a quantitative yield of 83 % as a brown oily material.

The ketone **36** (Scheme 24) underwent a reaction of methylation using the Tebbe reagent to produce a terminal methylene of compound **36a** (34 %). Tebbe reagents are often favoured in organic synthesis due their high yielding products in hindered substrates where other conditions fail or produce low yields[117]. The Tebbe reaction yielded the product **36a** as an oily brown material, which underwent an oxidation reaction using *m*-chloroperoxide producing the epoxide **36b** in an efficient yield of 87 %, as a white crystalline material. The benzyl group for the epoxide **36b** was deprotected using a standard hydrogenation reaction using palladium on carbon to produce the epoxide diol **37a** (Scheme 24). However, it was difficult to know if the reduction worked to produce the epoxide diol **37a**, because the hydrogenation may have been too vigorous and harsh, as the epoxide ring opened up. A number of different hydrogenations were attempted to find a selective set of reactions conditions, which only reduced the required benzyl groups, while keeping the epoxide ring intact. In all cases the reactions led to the epoxide ring opening[100].

The glycine derived bicyclic [3.2.1] orthoester **38** shown in SCHEME 24 was abandoned due too many reactions involved in the overall synthetic route. Also these synthetic steps would eventually produce a low monomer yield recovery, which was deemed unsatisfactory as high monomer mass is required in polymerisations. Furthermore, the long synthetic route would possess too many difficult synthetic issues in the scale-up synthesis, as well as being time consuming and costly (potentially expensive industrial scale-up synthesis). Another reason the synthetic

route was stopped, due to the deprotection of **37a** (Scheme 24) proved difficult to be addressed due to the competitive side reaction that occurred. Although as mentioned earlier, different reaction conditions were investigated for selective deprotection without much success.

### *2.2.2 Bicyclic [3.2.1] orthoester monomer derived from 3-methylene butan-1-ol*

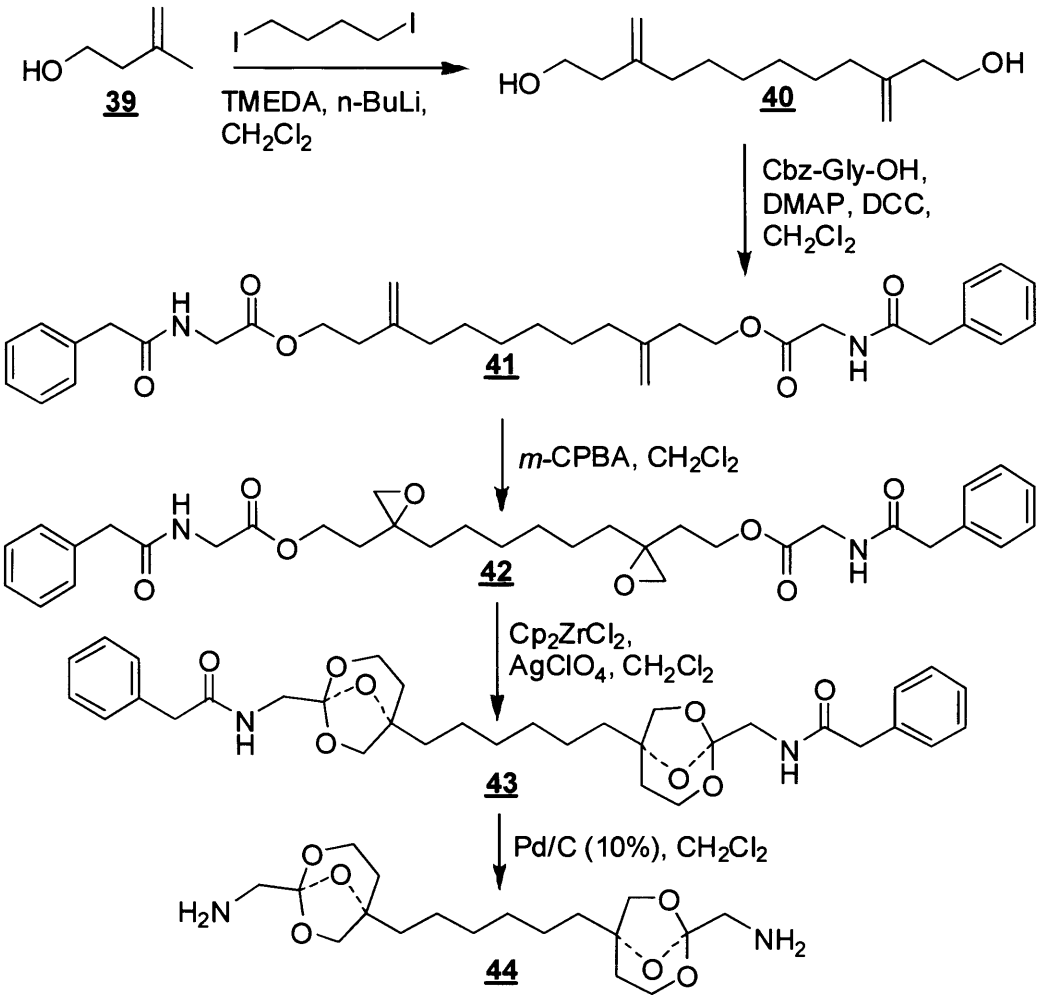
### *2.2.3 Di-substituted bicyclic [3.2.1] orthoester derived from 3-methylene butanl-1-ol*

The attempts that were made to prepare the bicyclic [3.2.1] orthoester monomer **38** (Scheme 24) gave us a better understanding of the chemistry involved. This included some synthetic issues, such as the length of the synthesis, and potential side and competing reactions occurring. Generally monomer **38** (Scheme 24) syntheses were inhibited due to a few synthetic difficulties that were highlighted previously. In order to achieve the goal of preparing a bicyclic [3.2.1] orthoester monomer, the synthetic approach outlined in Scheme 24 needed redesigning to incorporate and addresses the challenges and difficulties that were came across (section 2.4.0 'bicyclic [3.2.1] orthoester derived from amino acid epoxy ester).

The bicyclic [3.2.1] orthoester monomer **44** (Scheme 25) was designed in such a way to address the synthetic difficulty issues of preparing bicyclic [3.2.1] orthoester monomer **38** (Scheme 24).

The synthetic monomer preparation advantageous characteristics include:

1. Bicyclic [3.2.1] orthoester ring arrangement incorporating a high stability property, while delicate enough to undergo rapid degradation in slightly acidic pH environments within the host.
2. Fewer reaction steps (providing favourable scale-up conditions).
3. No complex deprotection chemistry needed or uncontrollable chemical reaction conditions (competing reactions).
4. Monomer design based on amino acid providing a better chance of the monomer being bioresorbable within the host.




**Scheme 25**: 3-methyl-buten-1-ol **39** derived bicyclic [3.2.1] orthoester **44** monomer synthesis.

As a starting point the 3-methyl-buten-1-ol **39** (Scheme 25) was used as it was readily available, in preparing the bicyclic orthoester monomer **44** (Scheme 25). Also disconnection chemistry yielded molecule **39** and bis alkyl halides. Furthermore, the chemistry of this molecule is well known (Figure 36). The alkylation and allylic halides chemistry of dilitho derivative of **40** (Scheme 25) has been known since 1974; and reactions with aldehydes are also known[118-120].

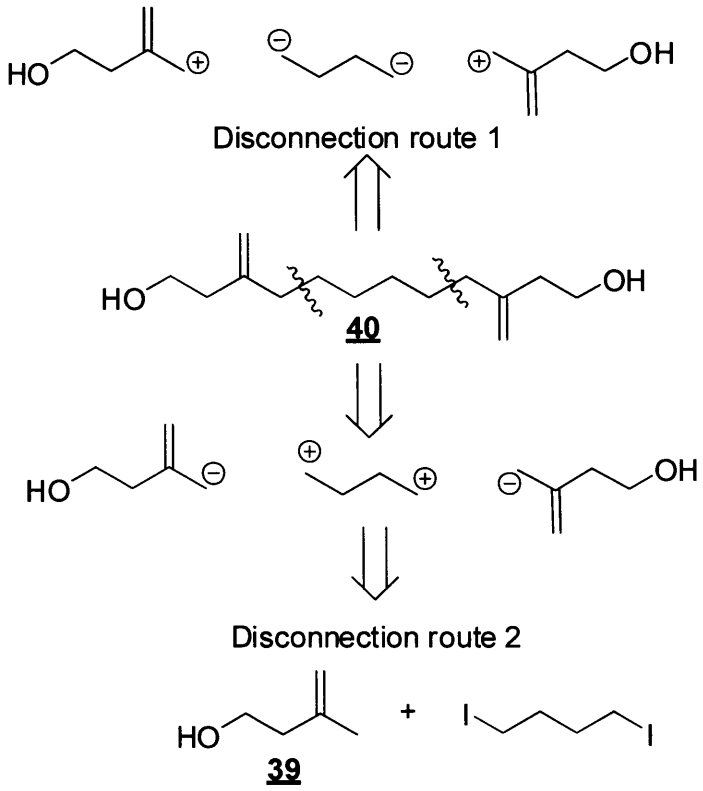



**Figure 36:** Disconnection of 3, 10-dimethylene-dodecane-1,12-diol molecule **40**.

Alkylation reaction of compound **39** (Scheme 25) with 1,4-diiodobutane using n-BuLi in excess TMEDA was undertaken to prepare the vinylic diol **40** (Scheme 25). This metalation reaction yielded no product as confirmed by $^{1}$H-NMR in the first attempt. Numerous experimental reaction conditions were attempted including: stiochiometry, concentration, temperature and reaction times were varied without any significantly different results. Moreover, an increased number of side-products was observed in each experiment. The most significant of these side-products was

isolated and identified spectroscopically as the vinyl ester **40a** (Figure 37). This may have been attributed to the di-anion generation during the metalation reaction, first the anion formed in the secondary terminal alcohol and second anion on the terminal methyl alpha to the vinyl group (Figure 37). The secondary terminal alcohol anion may have been more reactive as compared to the desired anion on the methyl alpha to vinyl group, therefore the reaction results in the ester being formed.

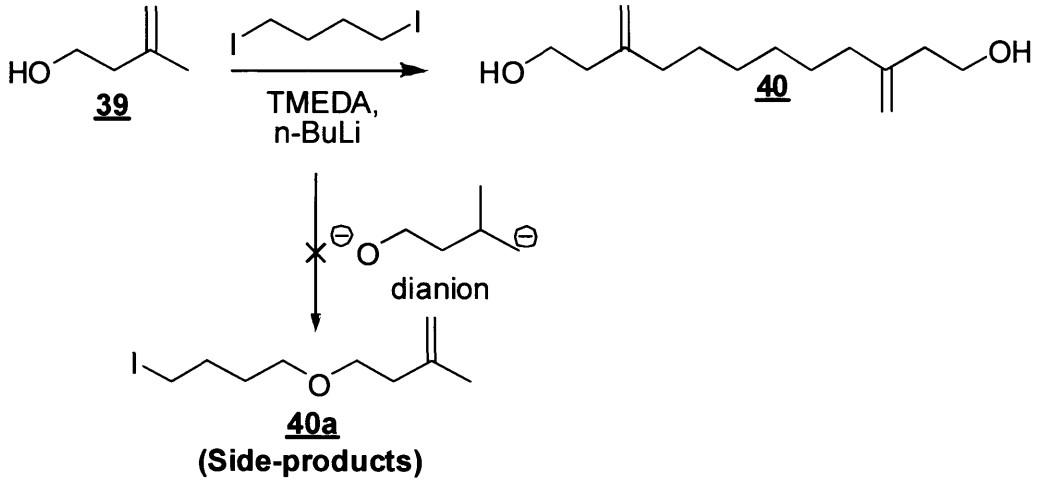


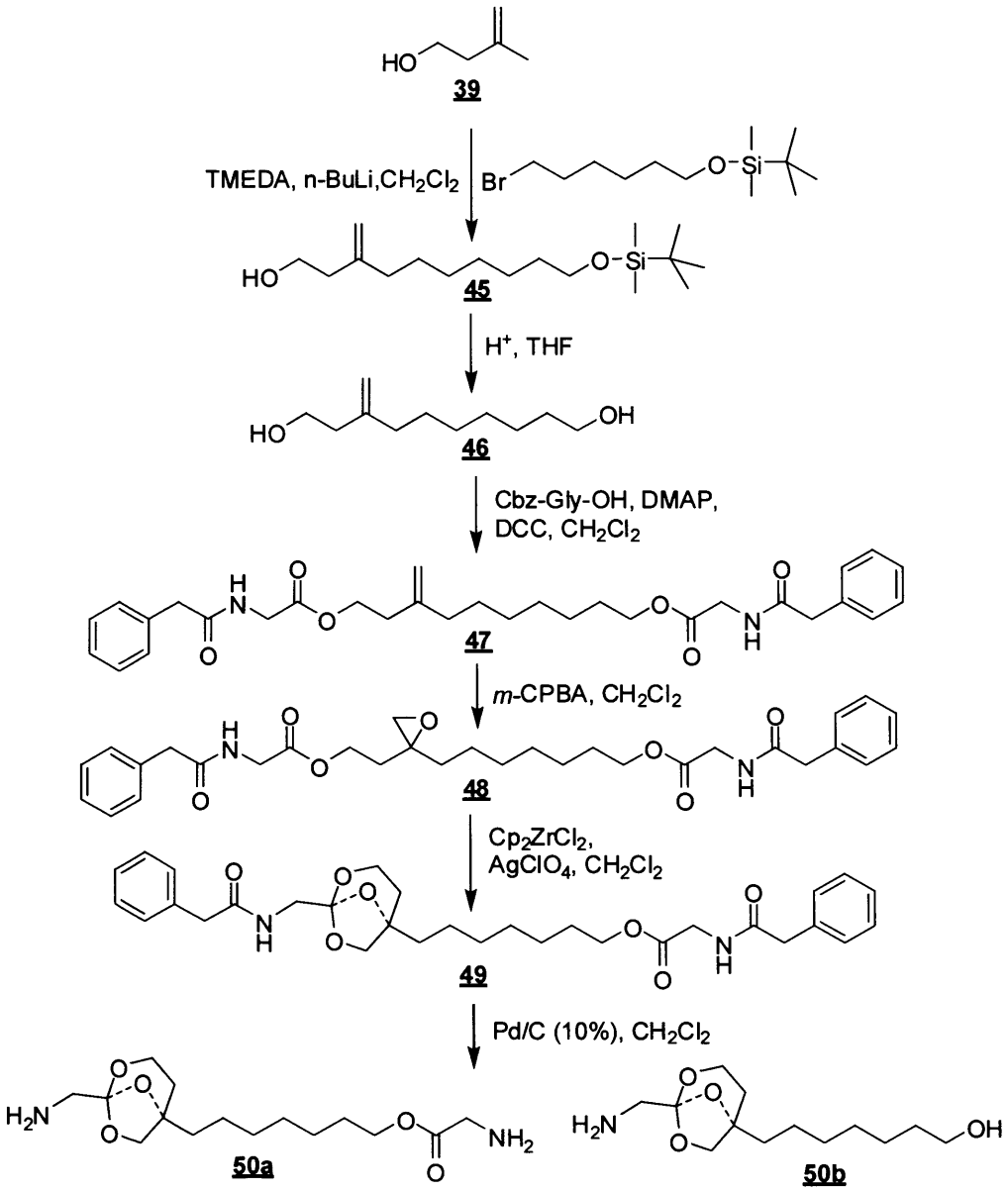

**Figure 37:** Ester **40a** as a side-product of the metalation reaction of 3-methyl-buten-1-ol **39**.

There were no examples of the symmetrical molecule **40** (Scheme 25) being prepared in the literature, or any related information to similar molecules that may provide us with an informative indication if the reaction was feasible. However, there was information of the mono substitution reaction, where only mono alkyl halides were involved. A study by Menges et al have prepared a series of alkylation of **39** (Scheme 25) with mono substituted allyl bromide. The alkylation was prepared using potassium hydrides and n-BuLi as the metalation conditions. This reaction condition was different from the reaction conditions that we previously attempted (TMEDA and n-BuLi) (for reactions of **40** (Scheme 25))[121]. Using this information

we were able to re-design the synthetic route (Scheme 25) to potentially prepare a bicyclic [3.2.1] orthoester based monomer. We hoped the new synthetic route would address the difficulties faced in the first step of the previous synthetic route, namely being the alkylation of vinyl alcohol **39** to prepare the molecule **40** (Scheme 25).

### *2.2.4 Mono-substituted bicyclic [3.2.1] orthoester derived from 3-methylene butan-1-ol*

The new synthetic route involved the use of a mono substituted alkyl halide in reaction step one (alkylation of vinyl alcohol **39** (Scheme 26)). Menges et al successfully have shown alkylation of vinyl alcohol **39** (Scheme 26) with mono substituted alkyl halides to prepare a series of alkylated 3-methylene butan-1-ol derived compounds[121]. A follow on study, by Yong et al reviewed Menges et al work and studied their own route of the alkylation of 3-methylene butan-1-ol dianion[122]. This involved the use of mono substituted alkyl halides[122]. Using this information formed the basis of our investigation to prepare a mono-substituted bicyclic [3.2.1] orthoester monomer **50a** or **50b** (Scheme 26) derived from vinyl alcohol **39** (Scheme 25).




**Scheme 26:** Mono-substituted and di-substituted bicyclic [3.2.1] orthoester monomer **50a** and **50b**.

The orthoester monomer **50a** or **50b** (Scheme 26) attempts to incorporate the potential advantageous characteristics as highlighted earlier of orthoester monomer **44** (Scheme 25). Furthermore, the orthoester monomer **50a** or **50b** have their own unique advantages including:

1. Initial reaction step has more potential to be successful as similar compounds have been described in the literature[121-123]. Particularly, reactions involving the starting molecule vinyl alcohol **39** (Scheme 26) have been described in

the literature.

2. When the precursor orthoester monomer molecule **49** (Scheme 26) is deprotected to generate the orthoester monomers **50a** and **50b** (Scheme 26), a unique set of monomers can potentially be prepared.

3. Monomer **50a** has two terminal secondary amines as the polymeriseable functionality, more like an A-A type monomer. Which can potentially be *co*-polymerised with commercially available B-B type monomers to prepare tailor made co-polymers for biomedical applications (see Chapter 3).

4. Alternatively, monomer **50b** has a terminal secondary amine and a terminal primary alcohol, thus potentially providing an A-B type monomer if further chemical modification to the orthoester monomer **50b** was undertaken.

We attempted for the first time to prepare and show an example of the synthesis of molecule **45** (Scheme 26). The synthesis involved dissolving vinyl alcohol **39** (Scheme 26) in ether and the addition of 1:1 stiochiometric ratio of n-BuLi for each reactive site of vinyl alcohol to generate a dianion, in presence of TMEDA. As the dianion of vinyl alcohol **39** was generated the solution turned yellow to orange in colour as reported by Yong et al[122]. To this solution, the relevant electrophile was added dropwise at -78 °C and left to warm to room temperature slowly overnight. The reaction did not produce the desired molecule **45** (Scheme 26) as identified through the $^{1}$H-NMR, where only the starting material was found. The experimental conditions were followed exactly as reported by Yong et al and Menges et al, including the same alkyl halide (bromide) as the leaving group was used[122].

It was assumed that the very reactive dianion species generated from the vinyl alcohol **39** (Scheme 26) may have reacted with a small amount of adventitious water or moisture. The reaction solvent ether was freshly distilled from calcium hydride and the reaction conducted under a flow of argon. To eliminate the possibility adventitious water interfering with the reaction, a slightly hygroscopic reaction solvent ether was changed to less hygroscopic hexane. Again the product was not formed and the crude product was predominately the starting material. Therefore the

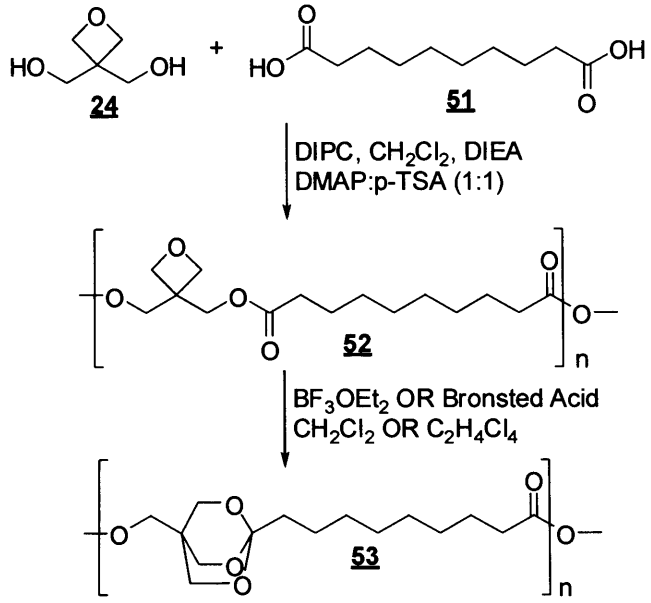
reaction was not purified beyond the initial $^1$H-NMR. The variable experimental condition that were studied include: stoichiometric ratio, concentration, temperature (-78 °C to room temperature) and reaction time (2 hours to 96 hours). None of these changes helped in the synthesis of molecule **45** (Scheme 26). Hexane as the reaction solvent caused the reaction mixture to turn into slurry, which was difficult to stir. Therefore, a further set of reactions was attempted using *co*-solvents. These *co*-solvents included slightly increasing the content of hygroscopic ether to the hexane as the major solvating agent, while minimising the introduction of moisture or adventitious water into the reaction system. The reaction mixture was a slurry in 100 % hexane dissolved with a co-solvent with a minimum of 10% ether content. However, these changes had very little effect in the product formation. Once again only the starting materials remained in the crude product mixture.

The difficult synthetic issues associated with step one of both synthetic routes (Scheme 25 and 26) could not be addressed. A number of experimental reaction conditions were investigated. The synthesis beyond reaction step one of both scheme 25 and 26 could not be over come in preparing the orthoester monomer **44**, **50a** and **50b** (Scheme 25, and 26). Both of these synthetic routes were abandoned and an alternative synthetic route of preparing an orthoester monomer that satisfies the specification as outlined in Chapter 1 (aims) and Chapter 3 were examined. For example even if the first reaction would have worked, its scalability would have a huge problem. Therefore, this would be an expensive reaction just for a monomer, it is essential to use a large quantity of the monomer in the polymerisation step. This would enable a better chance for the polymerisation being successful and obtaining high molecular weight polymers.

### *2.3.0 pH triggered rearrangement reaction used to prepare poly(ortho esters)*

Apart from preparing the poly(ortho esters) by polymerisation of the monomer with the degradable orthoester element embedded within the monomer prior to polymerisation. We have also prepared an alternative route for preparing poly(ortho esters). This route involves the preparation of a precursor polymer **52** (Scheme 27), which is then isolated. The isolated precursor co-polymer **52** undergoes a pH

triggered rearrangement reaction facilitated by an acid catalysed (triggered) intra-molecular rearrangement reaction to prepare the new poly(ortho esters) **53** (Scheme 27).




**Scheme 27:** pH triggered rearrangement reaction of precursor co-polymer **52** to prepare a poly(ortho esters) **53**.

The pH triggered rearrangement reaction technique was developed from our earlier work that was carried out as outlined in Chapter 3. The bicyclic [2.2.2] orthoester is prepared through an intra-molecular rearrangement reaction of an oxetane ring that is in beta position to a carbonyl ring (Figure 38). Therefore, using this required chemical functionality a precursor *co*-polymer was designed.

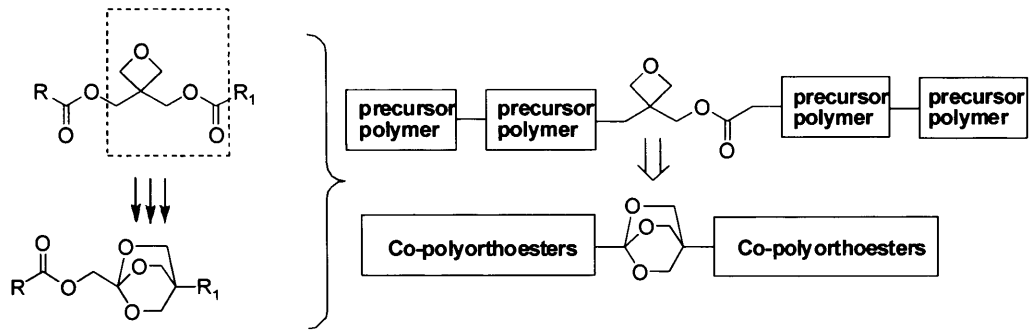



**Figure 38:** Chemical functionality requirements needed to prepare a bicyclic [2.2.2] orthoester based poly(ortho esters).

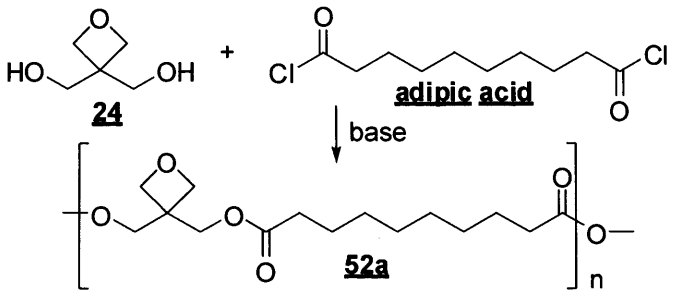
### *2.3.1 Synthesis of the precursor poly (oxetane esters)*

#### *2.3.1.1 Poly (oxetane esters) prepared by direct polycondensation using diacid chlorides.*

The oxetane diol **24** (Scheme 28) was polymerised with an activated diacid chlorides, particularly adipic acid in the presence of a base e.g. triethylamine (Scheme 28). The *co*-polymerisation yielded the poly (oxetane esters) **52a** (Scheme 28) in a low molecular weight (12, 000 g $mol^{-1}$ (2.34 PDI)). The *co*-polymer was an oil like material in appearance and mainly composed of oligomers (as identified in the GPC with THF as the solvent). It is widely accepted that preparing polyester with diacid chlorides often produce polymers that are low in molecular weight [14;124]. The potential issues that need to be addressed if successful polymerisation are to be achieved, includes heating reaction at high temperatures under complete anhydrous conditions. We attempted a number of reactions with varying experimental reaction conditions including:

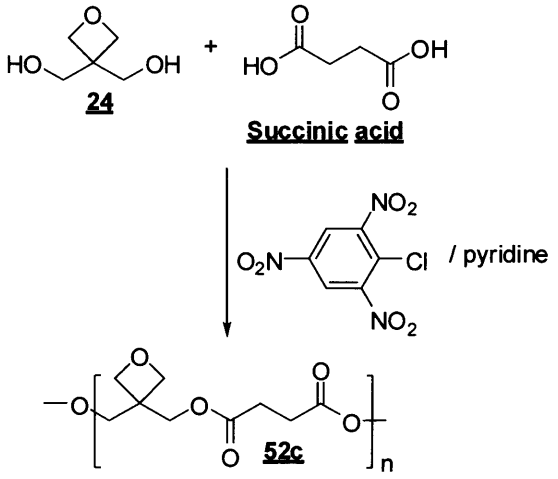
1. Heating the polymerisation at higher temperatures and under reduced pressure, such as conducting the reaction in a glass vacuum vessel.
2. The polymerisation solvent THF was freshly distilled (sodium/benzophenone) to make the solvent as anhydrous as experimentally possible.
3. A series of reactions with different bases such DIEA (Hunig's base), pyridine and dimethyl amino pyridine (DMAP) was examined.
4. Polymerisation reaction time was also varied from minimum of 2 hours to leaving overnight (while reducing the temperature (safety reasons)).

Varying the experimental reactions had very little effect in reaching high molecular weight poly (oxetane esters) **52a** (Scheme 28). Alternative polymerisation routes for preparing poly (oxetane esters) from oxetane diol and diacids were further examined.




**Scheme 28:** Poly (oxetane esters) **52a** prepared by a polymerisation of a oxetane diol **24** and a diacid chlorides (adipic acid).

A study by Tanaka et al have successfully polymerised a series of diols with a series of diacid using picryl chlorides as the catalyst in presence of 100 % pyridine as the reaction solvent phase[125]. A small library of *co*-polymers was generated with high viscosity. However, there was no mention in the article that the polymers produced were high molecular weight. It was mentioned that the polymerisation does proceed at ambient temperature, and does not require harsh reactions conditions. Tanaka et al reaction conditions which are less rigorous for the sensitive monomers, particularly oxetane diol **24** (Scheme 28). Oxetane diol **24** if placed in a harsh environment has a tendency for the oxetane ring to open and undergo a polymerisation reactions to form polyols[98]. Examples of these harsh reaction conditions include high temperatures or placing in acidic environments. We examined using the Tanaka *co*-polymerisation method to polymerise monomer **24** with succinic diacid to generate the poly (oxetane esters) **52c** (Scheme 29).




**Scheme 29:** Tanaka's method used to prepare poly (oxetane esters) **52c** [125].

After several attempts no poly (oxetane esters) **52** were prepared. A closer examination of the reaction mixture yielded the starting materials only, and there was no indication in the $^{1}$H-NMR of an ester forming. As previously, a number of reaction conditions were attempted to find a set of favourable polymerisation conditions that may prepare the poly (oxetane esters) **52c** (Scheme 29). These reaction conditions included:

1. Changing the stiochiometric ratio of picryl chlorides to succinic acids.

2. A series of reactions was under taken with varying the time of reaction from a minimum of 15 hours to continuing the reaction over a week period.

3. The temperature was adjusted from carrying reactions at 70 °C to conducting reactions at ambient temperatures.

There were no polymerisations occurring, as a result of changing the experimental reaction conditions using Tanaka's methods [125]. As previously, only the starting material remained. A stand alone polymerisation with oxetane diol monomer **24** and a diacid chloride (diglycolyl chloride) in pyridine as the solvent with no picryl chloride was attempted. The polymerising reaction mixture got slightly viscous and reaction mixture was directly used to precipitate the polymer out from hexane as the insoluble solvent. However, it was difficult to isolate a polymer. A very low molecular weight polymer may have been produced, which was difficult to analyse further.

#### *2.3.1.2 Precursor poly (oxetane ester) prepared through carbodiimide coupling route*

Polyesters and their *co*-polymers are can be produced by solution polycondensation using carbodiimide coupling of a diacid and a diol[25]. Few methods in the literature describe the carbodiimide coupling of an acid with an alcohol[126]. For efficient coupling and achieving high molecular weight polymers, the acid must be highly

activated and the unwanted by-product *N*-acylureas **54b** (Figure 39) must be suppressed[127]. Moore and Stupp room temperature polyesterification was the first example of producing high molecular weight polyesters from sensitive monomers, especially in the case of the oxetane diol **24** (Scheme 29)[128]. The success of the polyesterification was due to the suppression of the unwanted by-product *N*-acylureas **54b** formation (Figure 39).

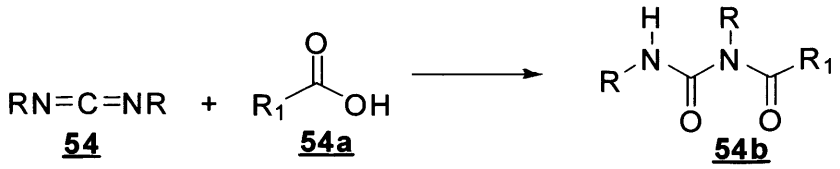
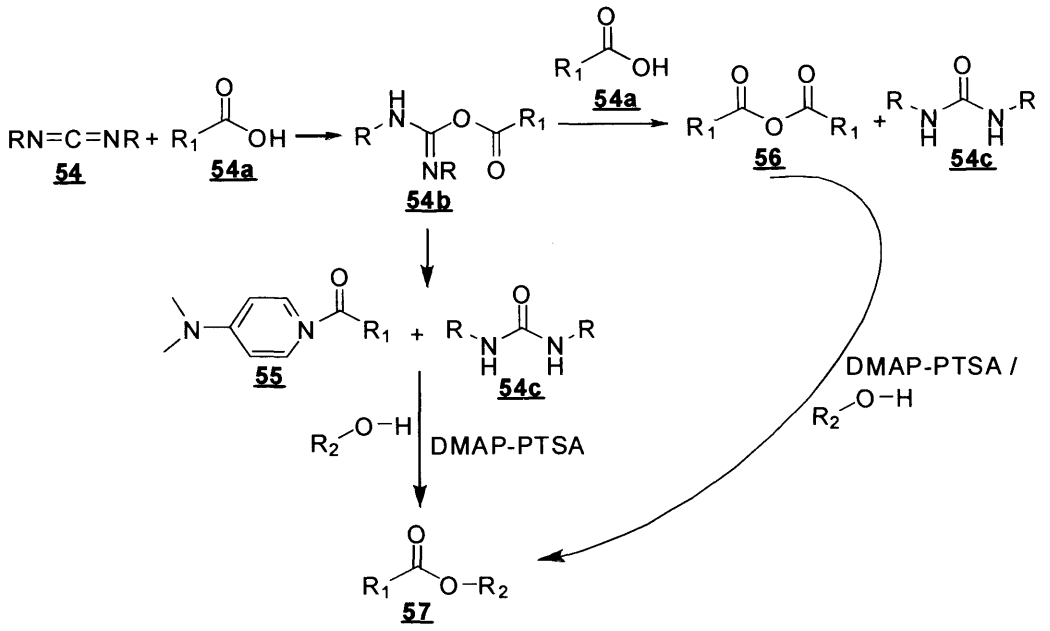
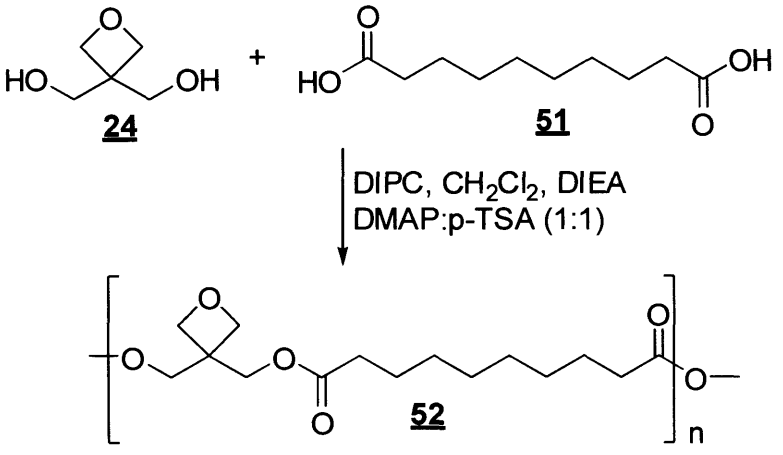



**Figure 39:** N-acylurea **54b** formation in an carbodiimide coupling reaction.

*N*-acylureas **54b** was suppressed by using a 1:1 complex of dimethylaminopyridine (DMAP) and p-toluene sulphonic acid (PTSA). The presence of hyperacylation DMAP produces the activated *N*-acylpyridinium **55**, while PTSA promotes a series of proton transfers in the formation of the urea (pathway A (Scheme 30)). Another possible mechanism (pathway B (Scheme 30)) suggested by Moore and Stupp is the formation of the anhydride **56**, which underwent a further nucleophilic reaction by the alcohol to generate the ester **57** (Scheme 30).




**Scheme 30:** Possible mechanism of an ester formation from a acid and a alcohol, that is assisted by an carbodiimide coupling as suggested by Moore and Stupp [128].




**Scheme 31**: Sebacic acid **51** co-polymerised with oxetane diol **24** to prepare poly (oxetane esters) **52**.

*Table 6*: Polyesterification of precursor co-polymer optimization study.

| Polymer | Solvent | Molecular weight (mol-1 x $10^3$)$^+$ | PDI |
|---|---|---|---|
| **P1** | DMF | - | - |
| **P2** | DMSO | 13.2 | 1.09 |
| **P3** | $CH_2Cl_2$ (20 % DIEA*) | 103.08 | 2.36 |
| **P4** | $CH_2Cl_2$ | 86.02 | 2.56 |
| **P5** | NMP | 15.56 | 1.28 |
| **P6** | $CH_2Cl_2$ / NMP (1:3) | 18.46 | 1.42 |

+ Conventional GPC calculation against PMMA in THF /

* DIEA used 20 % molecular equivalent

The precursor *co*-polymer was based on the polymerisation of the oxetane diol **24** with a diacid sebacic acid **51** to prepare the precursor *co*-polymer poly (oxetane esters) **52** (Scheme 27). The polymerisation reaction conditions that were examined was based on the Moore and Stupps room temperature method[128]. The precursor poly (oxetane esters) satisfied the required chemical functionality as shown in figure 4, as the oxetane ring was in the beta position to the carbonyl group. A series of sebacic acid reactions were examined as A-A monomer to be polyesterified with the oxetane diol **24** to prepare a series of poly (oxetane esters) (Scheme 31). The reactions were coupled with diisopropyl carbodiimide with varying reaction conditions (Table 6). This was done to find a set of polymerisation reaction conditions that produced a high molecular weight co-polymer(s). Diisopropyl carbodiimide as the coupling agent is often the preferred choice of coupling agent within the literature to prepare polyesters *co*-polymers, because the by-product (urea) can be isolated from the polymer[128]. As the urea is soluble in most organic solvents, whereas the large polymeric material is not. Therefore, the polymer can be isolated and separated.

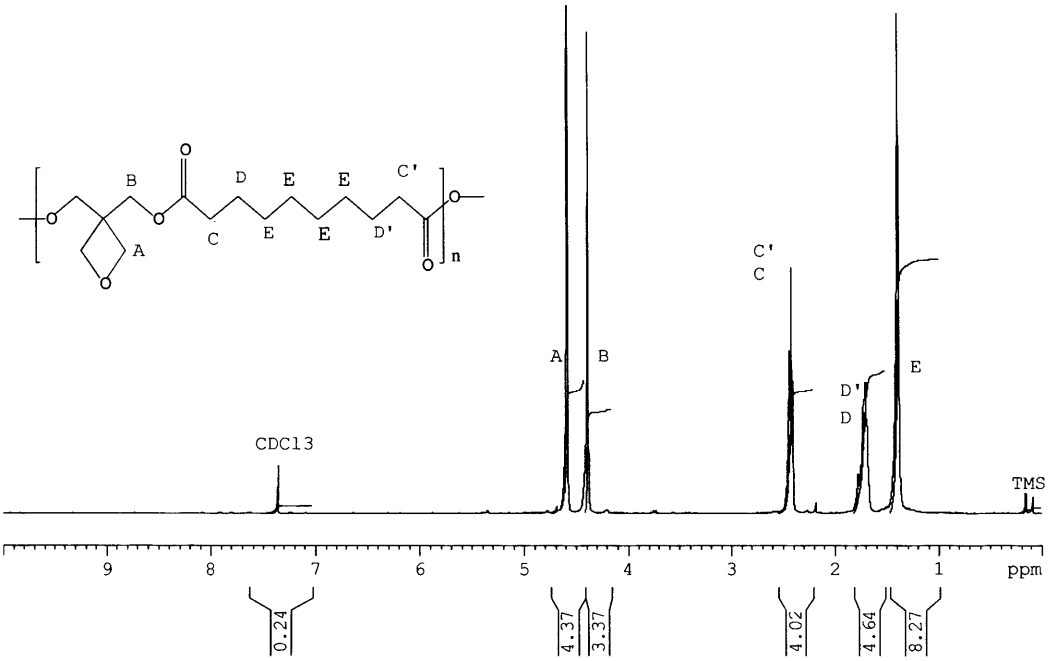



**Figure 40:** $^{1}$H-NMR of poly (oxetane ester) **53** in $CDCl_3$ at 22 °C.

From the optimization reactions, polymer **3** (**P3**) (Table 6) was obtained with the highest molecular weight. This reaction involved sebacic acid **51** *co*-polymerised to prepare the high molecular weight poly (oxetane ester) **53** (MW 103 Kda (g $mol^{-1}$ x $10^3$)). It was also noted that the high molecular *co*-polymers are prepared when the polarity of the solvent was reduced **P3** and **P4** (Table 6). Furthermore the use of the Hunig's base diisopropylethylamine increases the coupling efficiency **P3** (Table 6). The isolated poly (oxetane esters) **53** had an off white solid physical appearance and the polymer was also analytically pure, as shown by $^{1}$H-NMR (Figure 40). The infrared spectrum shows the oxetane ring absorption at peak 986.62 $cm^{-1}$. This is the characteristic signal of oxetane ring absorption within this region and it is generally accepted within the literature[129].

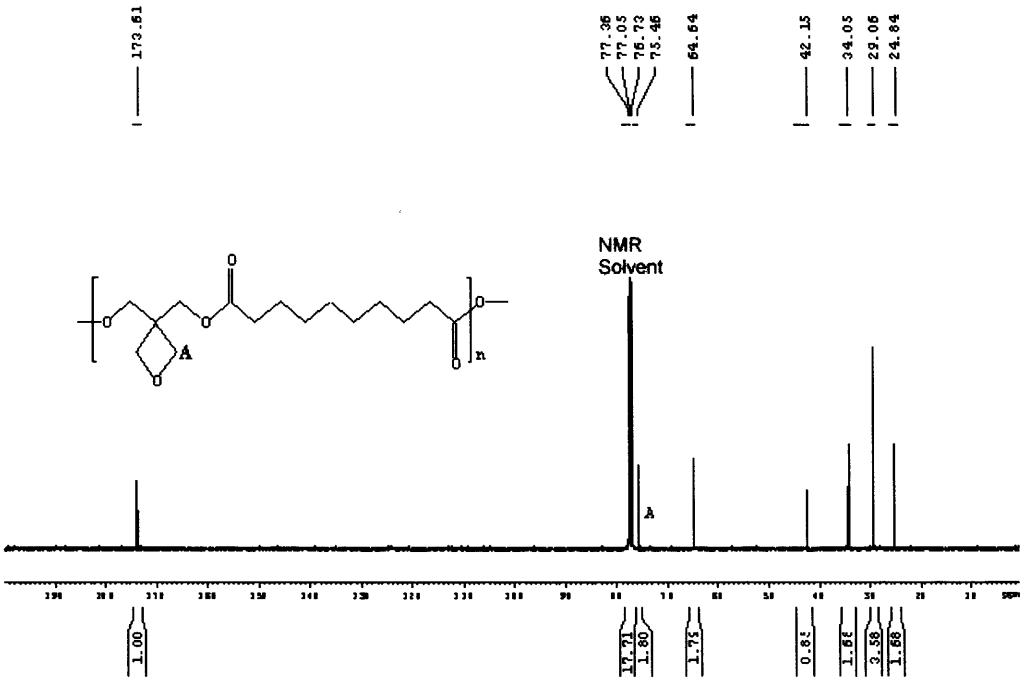



**Figure 41:** $^{13}$C-NMR of poly (oxetane esters) **53** in $CDCl_3$ at 22 °C.

### *2.3.2 pH triggered rearrangement reaction on precursor polymer to prepare poly(ortho esters)*

The precursor *co*-polymer **52** (Scheme 27) was found to undergo pH triggered rearrangement reaction facilitated by an acid catalysed (triggered) intra-molecular rearrangement reaction to prepare the new poly(ortho esters) **53** (Scheme 27). This is the first example within the literature that we have found. There are no examples of preparing highly degradable poly(ortho esters) using a pH triggered rearrangement reaction. The pH triggered rearrangement reaction involved precursor poly (oxetane ester) **52** being dissolved in a solution of $CH_2Cl_2$. To the polymeric solution the acid catalyst $BF_3OEt_2$ was added to facilitate a trigger to start the intra-molecular rearrangement reaction, leading to the poly(ortho esters) **53** (Scheme 27). The poly(ortho esters) **53** that are prepared are cross-linked materials with some unique properties. These unique properties open new doors for biomedical application were a potentially highly degradable material with the required good mechanical properties[25;34]. Such unique properties can potentially be applied in the field of drug delivery, where hydrogel like material properties are required[34]. The cross-linked highly degradable polymer matrix has the potential to be used in drug delivery for

controlled release applications[34]. Because, the poly(ortho esters) incorporation provided the polymer matrix with the ability to potentially undergo degradation predominately characterised by surface erosion processes[34;68]. Existing poly(ortho esters) reported within the literature follow this behaviour generally, and have been studied by many research groups[114].

***Table 7***: The effect of catalyst concentration and temperature on the degree of poly(ortho esters) conversion.

| Entry[a] | Catalyst Con[c] (% mol eq) ( solvent dilution) [b] | Temperature (°C) | Orthoester Conversion[c] (%) | Physical Appearance |
|---|---|---|---|---|
| **P7A** | 3 (10) | -78 | - | starting material |
| **P7B** | 3 (10) | -30 | - | starting material |
| **P7C** | 3 (10) | 0 | 19 | cross-linked |
| **P7D** | 3 (10) | room | 10 | cross-linked |
| **P7E** | 1 (10) | room | 23 | cross-linked |
| **P7F** | 10 (10) | room | insoluble | cross-linked |
| **P7G** | 30 (10) | room | insoluble | cross-linked |
| **P7H** | 3 (5) | room | insoluble | cross-linked |
| **P7I** | 3 (15) | room | 18 | cross-linked |
| **P7J** | 3 (20) | room | 21 | cross-linked |
| **P7K** | 3 (50) | room | 26 | cross-linked |

[a] Experimental procedures (A) are described in the Experimental Section. [b] 100 mg equivalent monomer ratio in variable solvent, temperature, and stirred overnight. [c] Conversion ratio calculated from $^1$H-NMR.

We have shown that a pH triggered rearrangement reaction can be performed on a precursor poly (oxetane ester) **52** (Scheme 27), to prepare the poly(ortho esters) **53** (Scheme 27). However, this new method of preparing poly (ortho esters) have produced polymers that are cross-linked materials. This led us to examine a series of reactions conditions to address the cross-linking of the polymers and thus prepare a linear polymer instead. In order to prepare a linear poly (ortho esters), a series of optimization reactions was under taken on these pH triggered rearrangement reaction (Table 7).

The optimization polymerisation study involved the effect of the reaction catalyst concentration, temperature and dilution order of the reaction. The general pattern that emerged from the study, was that in all cases a cross-liked POE **53** (Scheme 27) was prepared. It was clearly evident from the optimization study that the intra-molecular rearrangement reaction between the carbonyl group and oxetane ring in the beta position away from the carbonyl group, was difficult to control. We assumed that the neighbouring precursor polymers reactive sites and attacking species may have interrupted and reacted with some parts of the poly(ortho esters) **53** generated. Eventually leading to a networked based Poly(ortho esters) (cross-linked) being prepared. We put in place different counter active measures to inhibit this polymer network from occurring with little success, particularly the diluting of the reaction solution (Table 7). We also used a technique used by Heller et al on the preparation of the third generation of the existing poly(ortho esters) (see chapter 1). Heller et al used a small molar percentage of iodine to stabilize the intermediate cation formed. This intermediate cation namely being on the carbon II as shown in Scheme 22. However, with the addition of this iodine cation stabiliser, the reaction was still difficult to control, thus producing the cross-linked poly(ortho esters) **53**.

To control the cross-linking during these intra-molecular rearrangement reactions we opted to change the current acid catalyst $BF_3OEt_2$ (trigger for pH triggered rearrangement reaction) to weaker acids (Table 8). A series of optimization reaction conditions were investigated as shown in Table 8. From the study the TFA acid catalyst not only catalysed the intra-molecular rearrangement but also the acid degraded the polymeric chain. Therefore, reducing the molecular weight, an example being the polymerisation of **P8D** (Table 8), where 92 % degradation occurred. Other acid catalysts that were examined include PTSA and camphor sulphonic acid (CSA). Both of these catalysts did not produce results that were meaningful, for example as the linear poly(ortho esters) **53** conversion from the precursor poly (oxetane esters) **52** occurred an almost similar rate of polymer degradation occurred.

***Table 8***: The table shows the effect of different catalysts that were examined in the preparation of poly(ortho esters) **53**.

| Entry[a] | Catalyst & Con[c] (%)[b] | Temperature (°C) | Orthoester conversion[c] (%) | Polymer degradation (%) [d] |
|---|---|---|---|---|
| **P8A** | p-TSA (5) | 80 | 66 | 59 |
| **P8B** | p-TSA (5) | 80 | 100 | 76 |
| **P8C** | CSA (5) | 80 | 92 | 81 |
| **P8D** | TFA | 80 | 100 | 92 |
| **P8E** | p-TSA (3) | room | 0 | 0 |
| **P8F** | p-TSA (3) | 80 | 48 | 64 |
| **P8G** | p-TSA (3) | 110 | 66 | 29 |
| **P8H** | p-TSA (3) | 120 | 46 | 21 |
| **P8I** | p-TSA (3) | 130 | 51 | 50 |
| **P8J** | p-TSA (3) | 150 | 58 | 60 |
| **P8K** | p-TSA (1)[+] | 120 | 13 | 35 |
| **P8L** | p-TSA (3)[+] | 120 | 35 | 31 |
| **P8M** | p-TSA (5)[+] | 120 | 59 | 66 |

[a] Experimental procedures (A) are described in the Experimental Section. [b] 100 mg equivalent monomer ratio in variable solvent, temperature, and stirred overnight. [c] Conversion ratio calculated from $^1$H-NMR. [d] Calculated using GPC (THF as solvent) against polystyrene standards. [+]Reaction run time of 40 min.

Through the pH triggered rearrangement reaction technique we have shown novel synthetic routes of preparing the widely studied poly(ortho esters), which have not been reported previously. These poly(ortho esters) **53** (Scheme 27) were prepared either as a cross-linked poly(ortho esters) or a linear Poly(ortho esters) and both open doors for potential novel biomedical applications. Furthermore, the poly(ortho esters) **53** synthetic routes shown within this chapter further complements the new synthetic route of preparing other new poly(ortho esters) as reported in Chapter 4. The other poly(ortho esters) reported in chapter 4 were prepared through the orthoester monomer embedded *co*-polymerisation with commercially available monomers to the poly(ortho esters).

# General discussion

This thesis is concerned with the synthesis of degradable acid labile *co*-polymers. The acid labile polymers systems that were examined included (1) polyacetals and (2) poly(ortho esters). As for poly(ortho esters), there is a need for better synthetic methods to prepare these polymers more easily without the need of stringent anhydrous conditions. Furthermore, these poly(ortho esters) are required to be prepared with more broad structural variation, and in a more cost effective manner.

Pendent functionalised polyacetals derived from PEG and tyrosine derived monomer diols have been prepared and their structure activity relationships determined. A smaller size alkyl chain on the tyrosine derived monomer diol increased the rate of degradation of these polyacetal libraries. While the larger pendent alkyl groups have to some extent decreased the rate of degradation, presumably due to an increase in hydrophobic properties of these type of polyacetals. Therefore, allowing less water uptake into the polymer matrix for degradation to occur with more ease.

The second acid labile polymer system that was investigated included the poly(ortho esters). The poly(ortho esters) strategy involved the preparation of a novel stable orthoester monomers. The key aspect was to embed the orthoester within the monomer while providing orthogonal polymerisation functionality. A number of orthoester monomers were prepared with varying properties e.g. stability, purity, and number of synthetic steps. Of the many synthetic routes that were examined, the bicyclic [2.2.2] orthoester derived from the naturally occurring metabolite phenyl acetic acid was the most stable and efficient. This synthetic route attempted to address the synthetic limitations for the preparation of existing poly(ortho esters) and it is believed to be the first such example. The stabile symmetrical bicyclic [2.2.2] orthoester monomer was used to prepare the new poly(ortho esters). The bicyclic orthoester [2.2.2] ring arrangement provided the monomer with rigidity, therefore enabling a pure solid monomer to be prepared in three synthetic steps. This approach provided a more efficient polymerisation reaction that requires less stringent polymerisation reaction conditions then existing literature examples for preparing

poly(ortho esters). Furthermore, this synthetic approach provided two new examples of poly(ortho ester) *co*-polymers including poly(orthoester carbonates) and poly(orthoester urethanes).

The second broad strategy examined the synthesis of a hydrolytically stable precursor poly (oxetane esters). This precursor polyesters was prepared using a carbodiimide coupling reactions enabling a high molecular weight *co*-polymer to be prepared. The precursor *co*-polymer underwent a pH triggered rearrangement reaction within the polymer mainchain to prepare orthoester moieties in the polymer mainchain. This strategy provided another new synthetic example of preparing the acid labile poly(ortho esters), where the degradable element is not formed in the polymerisation step. This is an alternative approach to existing way of preparing acid labile polymers, particularly poly(ortho esters). Furthermore, this concept of forming the degradable element embed within the monomer with orthogonal polymerisation functionality that is co-polymerised can potentially be used to prepare other degradable polymers.

# Chapter 6

## *Materials & methods*

# Chapter 6

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# *Materials and Methods*

## *1.0 Materials and Instruments*

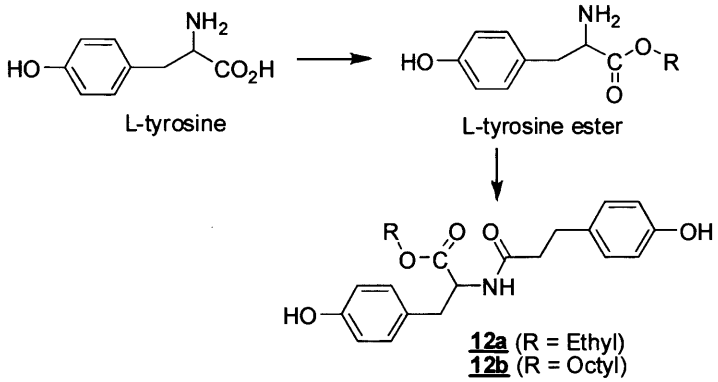
Anhydrous solvents were freshly distilled from either: sodium and benzophenone, $P_2O_5$ or $CaH_2$. All reactions were performed in oven-dried glassware under argon atmosphere.

All chemicals and anhydrous solvents were purchased from either Aldrich, Fluka, Lancaster, Avocado, Acros or Sigma chemical companies. IR spectra were recorded on an Avatar 380 FTIR spectrometer (Nicolet instruments) and analysed using OMNIC v. 5.0 software. NMR spectra were recorded in $CDCl_3$ unless stated otherwise on a Bruker AM 400 MHz spectrometer and are reported in ppm relative to tetramethylsilane ($\delta$). Data are reported as follows: chemical shift, multiplicity (s= singlet, d= doublet, t = triplet, q = quartet, m = multiplet, br = broad), integration and coupling constants. Analytical TLC used Merck silica gel 60 F-2254 plates, and flash chromatography on $SiO_2$ (otherwise stated) was used to separate and purify crude reaction mixtures. Spots were visualised using iodine crystal chambers, phosphomolybdic acid, UV light, and potassium permanganate. Polymer molecular weights were determined using a Gel Permeation Chromatography (GPC), in DMF (1 % LiCl) using two Viscotek GMHHR-M columns at 70 ° C. Viscotek triSec software was used to calculate the molecular weight characteristics and PMMA standards (102 500, 58 700, 32 800, 10 900, 5 090 and 1 960 Da) were used as calibrants. Conditions: sample preparation 3 mg $ml^{-1}$; flow rate 0.50 ml $min^{-1}$, loop seize 20 µl; injection volume 50 – 70 µl, detector refractive index (Gilson 133®). The glass transition temperature was determined with a Perkin – Elmer Pyris Diamond Differential Scanning Calorimeter (Perkin – Elmer instruments) under nitrogen. The DSC was temperature calibrated using indium as a standard and was controlled and the data analysed with Pyris 5.0 software. Thermally modulated temperature DSC (TMDSC) was conducted using StepScan – DSC ™ (Perkin – Elmer Instruments).

## 2.0 *Work as described in Chapter 2*

***Representative ter-polymerisation procedure.***

***L-tyrosine derived diphenolic monomer* <u>12a</u> *and* <u>12b</u>**




The L-tyrosine derived monomer diol <u>12a</u> (<u>12b</u>) were prepared in two steps following the procedure described by Kohn *et al* [130]. The first step involved preparation of the L-tyrosine ester.

To a stirred solution of L-tyrosine (10 g, 55 mmol) in either ethanol or octanol (75 ml) at 0 °C was added thionyl chloride (4.4 ml, 60.7 mmol, 1.1 eq) drop-wise over 10 min. The mixture was heated to 70 °C for 18 hrs and cooled to ambient temperature. The crude product was isolated by precipitation into rapidly stirring ether (200 ml), filtered and dried in a vacuo. The spectroscopic data were in agreement with literature observation, therefore only a $^{1}$H-NMR spectrum was taken, the L-tyrosine ethyl ester was isolated with the yield of 10.99, (96 %).

$^{1}$H-NMR (400 MHz; DMSO): δ (ppm) 1.12 (t, 3H), 3.05 (m, 2H), 4.10 (m, 3H), 6.71 (d, 2H), 7.01 (d, 2H), 8.05 (bs, 2H), 9.43 (s, H). $^{13}$C-NMR (100 MHz; DMSO): δ (ppm) 13.7, 35.2, 53.4, 61.4, 115.2, 124.4, 130.3, 156.5, 169.1.

The L-tyrosine derived monomer diol <u>12a</u> was synthesised by carbodiimide coupling reaction of the L-tyrosine ethyl ester (8.0 g, 38.2 mmol) with hydroxyphenylpropionic acid (6.36g, 38.2 mmol) and 1-hydroxybenzotriazole hydrate (0.51 g, 3.8 mmol, 0.1 eq) and dissolved in acetonitrile (70 ml). To solution 1-(3-dimethylaminopropyl)-3-ethyl-carabodiimide hydrochloride (7.33 g,

38.2 mmol) was added. The reaction was stirred for 18 hrs and dried in a vacuo. The resulting oil was dissolved in ethyl acetate (200 ml), washed with potassium carbonate solution (10 % aq (20 ml x 3)), HCl (0.01M (20 ml x 3)) and finally with brine (20 ml). The organic phase dried over magnesium sulphate, filtered and dried in a vacuo. The crude product was purified via flash chromatography on silica (ether:hexane (4:1)) $R_f = 0.31$, to give a white solid (7.7 g (45 %)).

$^1$H-NMR (400 MHz; DMSO): δ (ppm) 1.11 (t, 3H), 2.31 (m, 2H), 2.62 (m, 2H), 2.78 (m, 2H), 4.02 (m, 2H), 4.36 (m, H), 6.64 (m, 4H), 6.97 (m, 4H), 8.19 (d, H), 9.13 (s, H), 9.22 (s, H).

$^{13}$C-NMR (100 MHz; DMSO): δ (ppm) 13.9, 29.4, 35.5, 36.9, 53.8, 60.2, 114.9, 128.9, 130.4, 131.1, 155.4, 155.8, 171.5, 172.1.

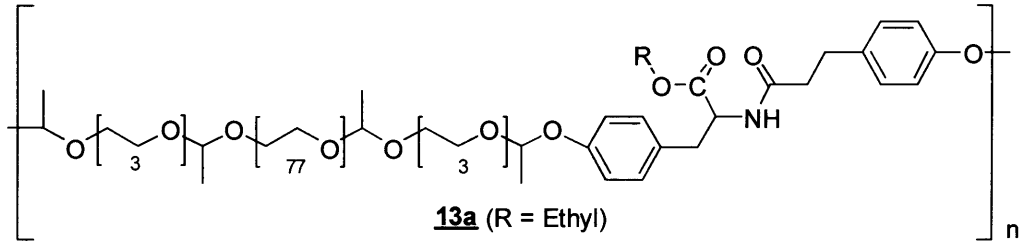
***L-tyrosine derived diphenolic monomer diol <u>12b</u>***

The same reaction procedure as **<u>12a</u>** were used for the synthesis of compound **<u>12b</u>**. The crude product was purified via flash chromatography on silica (ether:hexane (4:1)) to give a white solid (9.2 g (54 %)). $R_f = 0.39$.

$^1$H-NMR (400 MHz; $CDCl_3$): δ (ppm) 0.87 (t, 3H), 1.35 (m, 10H), 1.61 (m, 2H), 2.37 (m, 2H), 2.73 (m, 2H), 2.92 (m, 2H), 4.09 (m, 2H), 4.78 (m, H), 6.08 (d, H), 6.6 – 6.9 (m, 8H), 6.94 (bs, 2H).

$^{13}$C-NMR (100 MHz; $CDCl_3$): δ (ppm) 14, 22.6, 25.8, 28.4, 29.1, 30.4, 31.7, 36.9, 38.1, 53.4, 66.0, 115.6, 115.7, 126.9, 129.4, 130.3, 131.8, 154.5, 155.3, 172.1, 172.8.

***Synthesis of polyacetals <u>13a</u>***




**<u>13a</u>** (R = Ethyl)

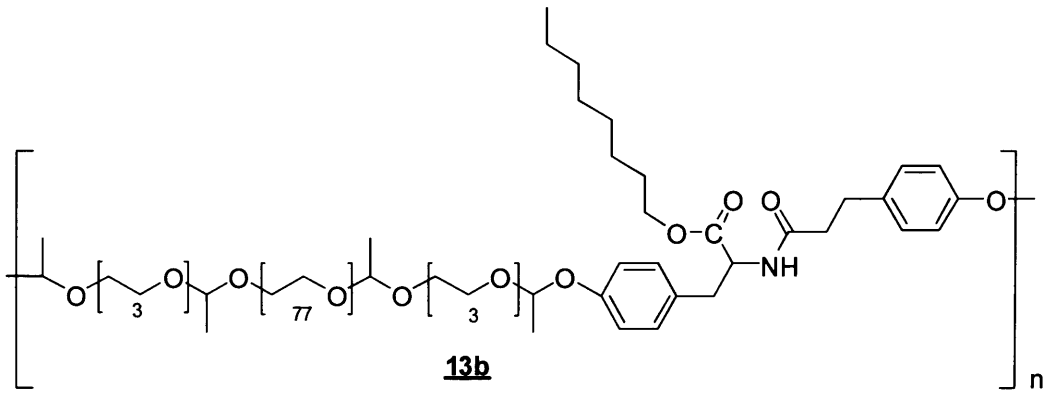
Poly(ethylene glycol) **<u>9</u>** ((Mp = 3 400 g mol$^{-1}$) (10.202 g, 3.00 mmol, 0.57 eq) and p-toluene sulfonic acid monohydrate (39 mg, 0.21 mmol, 0.04 eq) were dried by

stirring *in vacuo* at 80 °C for 16 hrs. The reaction flask was re-weighed enabling subsequent reagent stoichiometries to be corrected. The ethyl ester L-tyrosine diol monomer **12a** (0.784 g, 2.20 mmol, 0.43 eq) was added to another oven dried flask. Which was then sealed and purged with nitrogen and dry THF (5 ml) was injected and once the mixture dissolved, the solution was cooled to -78 °C and freeze dried *in vacuo* for 2 hrs. The residue was re-dissolved in THF (5 ml) and transferred *via* a cannula to the reaction mixture containing PEG. The second flask containing the L-tyrosine monomer diol **12a** was washed with THF (2 x 2 ml) and transferred to the reaction vessel containing the PEG mixture. Freshly distilled triethylene divinyl ether **10** (distilled from $K_2CO_3$) (1.06 ml, 5.19 mmol, 1.00 eq) was injected drop-wise to the rapidly stirring reaction mixture. The reaction was placed in an oil bath at 35 °C for 3 hrs, a further THF (2 ml) was added if the reaction got to viscous to stir. An additional divinyl ether **10** (0.1 ml) was then added drop-wise over for 20 min. Triethylamine (0.2 ml) was then slowly added drop-wise to the polymerisation to stop the reaction. The polyacetal **13a** was isolated by precipitation at ambient temperature by pouring into rapidly stirring diethyl ether (400 ml).

$^1$H-NMR (400 MHz; $CDCl_3$): δ (ppm) 1.22 (t, 3H), 1.28 (m, 3H), 1.45 (m, 3H), 2.41 (m, 2H), 2.85 (m, 2H), 2.98 (m, 2H), 3.40 – 4.00 (m, 2H (PEG)), 4.13 (m, 2H), 4.36 (m, 1H), 4.76 (m, H (x2)), 5.94 (m, 1H), 6.60 – 7.20 (m, 8H).

$^{13}$C-NMR (100 MHz; $CDCl_3$): δ (ppm) 14.1, 19.5, 20.0, 30.5, 37.0, 38.2, 53.8, 61.4, 68.0 – 70.9, 99.5, 99.8, 117.2, 117.4, 129.3, 130.2, 134.0, 134.1, 155.3, 155.8, 171.5 - 171.6.

***Synthesis of polyacetals* 13b**




**13b**

Integrals quoted treat the PEG and the L-tyrosine derived diphenolic monomer subunits separately; their relative values are listed in Table 1 (Chapter 2).

$^{1}$H-NMR (400 MHz; $CDCl_3$): δ (ppm) 0.87 (m, 3H), 1.27 (m, 3H), 1.46 (m, 3H), 1.59 (m, 2H), 2.43 (m, 2H), 2.85 (m, 2H), 2.99 (m, 2H), 3.40 – 3.95 (m, 2H (PEG)), 4.09 (m, 2H), 4.78 (m, 1H (x2)), 5.38 (m, 1H), 5.91 (m, 1H), 6.6 – 6.9 (m, 8H).

$^{13}$C-NMR (100 MHz; $CDCl_3$): δ (ppm) 14.0, 19.5, 20.0, 22.5, 25.8, 28.4, 29.1, 30.5, 31.7, 37.1, 38.2, 53.1, 65.6, 69.3 – 70.8, 99.6, 99.8, 117.3, 117.4, 129.1, 130.2, 134.0, 134.2, 155.3, 155.8, 171.4, 171.6.

***Thermal analysis***

Conventional DSC was conducted by first under-taking a two heating and cooling cycles to remove thermal history. This was achieved by heating a sample of the representative polymer (5 – 15 mg) from – 50 °C to 100 °C at a rate of 20 °C per min, and then cooling back from 100 °C down to – 50 °C at the same rate. Once the thermal history have been removed, the data was collected using the same procedure.

Thermal modulated DSC (TMDSC) analysis was conducted over the same temperature range of – 50 °C to 100 °C. However, the samples were heated in a step-wise configuration with steps of 2 °C and then holding at the new temperature for 4 min. This step procedure was repeated up to 100 °C. A blank run was conducted for data analysis, which was subtracted from the data collected of the sample, to produce a true and accurate value. The resultant difference curve gave the measured thermal parameters.

***Contact angle measurements***

Measurements were conducted on a KSV Cam200 contact system. Using a ultra-pure water, a drop of the water was applied to the surface of the sample and the angle between the substrate and the surface of the drop measured *via* the CCD camera and associated software. Three sample measurements were taken (n=3) and the mean from data was used for the analysis.

*Cell attachment study of polyacetals*

A borosilicate glass cover-slip was dip coated (x 3) in a polymer solution (10 – 15 mg in $CH_2Cl_2$ (10 ml)), and left over night (16 hrs) to evaporate under anhydrous condition. The polymer coated cover-slips were sterilised using radiation under a ultra-violet light source for 1 hr, before use. Three coated cover-slips of each of the polymer (n = 3) was used in the assay. Cells (MG63) were calculated for 72 hrs in Gibco® (UK) growth medium D-MEM containing a 1 000 mg $L^{-1}$: glucose, L-glutamine, 25 mM hepes, pyruvate, 5 ml penicillin streptomycin and 50 ml foetal bovine serum. A drop of tissue culture was seeded (1 x $10^4$ cells $cm^{-2}$) onto the surface of each of the cover-slips in a 24 well tissue culture plate and incubated for 4 hrs. After a fresh 2 ml of growth medium were added and incubated for a total for 6 or 24 hrs accordingly. The cell attachments were observed by scanning electron microscopy (SEM).

*SEM preparation*

The medium was removed and the cover-slips were fixed with 3 % glutaraldehyde in 0.1 M cacodylate for 30 min at ambient temperature. The fixative was removed and the cover-slips were dehydrated by a series of ethanol washes (20, 50, 70, 90 and 100 % (x 2)). This was followed by hexamethyldislazine (SPELL) wash and removed, then left to evaporate for 12 hrs. The evaporated cover-slips were metal coated (gold) with a Polaron E5000 sputter coater, and then observed by SEM (Cambridge 90B).

*Degradation study of the polyacetals*

The degradation stock solution was prepared by dissolving 100 mg of polyacetals in 20 ml of the buffer solution (0.1 mol $L^{-1}$ at pH 7.4, 6.5 and 5.5) to give a final polyacetal solution concentration of 5 mg $ml^{-1}$. The buffer solutions at the different pH values (0.1 M) were prepared from the stock buffer solution A and buffer solution B (each at 0.2 M) as follows:

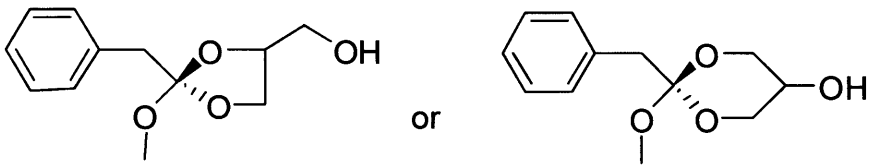
pH 7.4 buffer: 19.0 ml A + 81.0 ml B (the pH was adjusted and made up to 200 ml)
pH 6.5 buffer: 68.0 ml A + 31.5 ml B (the pH was adjusted and made up to 200 ml)

pH 5.5 buffer: 93.5 ml A + 6.5 ml B (the pH was adjusted and made up to 200 ml) The pH was adjusted with either 0.1 M HCl or 0.1 M NaOH to 7.58, 6.52 and 5.33.

The prepared polyacetal solutions (20 ml) were filtered through HPLC (0.2 µl) syringe filters. The polyacetal solutions were incubated at 37 °C and then were shaken gently throughout the degradation period. Aliquots of 0.5 ml of the polyacetal degradation solutions were removed at set time intervals (0, 6, 24, 48, 72, 99 hrs). These samples were frozen in liquid nitrogen, freeze dried under vacuum, and re-dissolved in 0.5 ml DMF for GPC analysis. The degradation analysis was conducted by GPC. The GPC elutograms were calibrated conventionally to PMMA standards, and the calibration was checked daily throughout the experiment using a PEG standard (3 400 Da).

## *3.0 Work as described in Chapter 3*

***4-Hydroxy (phenyl acetic) acid orthoester monomer synthesis***




**15a** **15b**

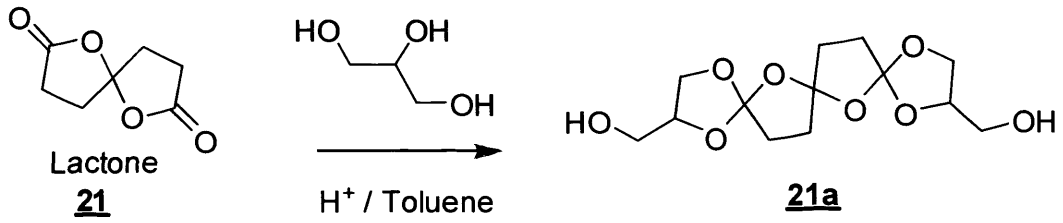
A stirred solution of trimethyl orthobenzoate (2 ml, 11.6 mmol) in toluene (15 ml), was fitted with a Dean-Stark apparatus and a $CaCl_2$ guard. To the solution, freshly distilled glycerol (0.85 ml, 11.6 mmol) was added by syringe followed by a drop of conc. $H_2SO_4$. The reaction was refluxed until theoretical amount of water collected, then the reaction was neutralized with triethylamine (0.5 ml). The solution was filtered through celite and concentrated in a vacuo. The residue was chromatographed on silica (pre-treated with 1 % triethylamine) ((ethyl acetate / hexane (4:1)) with 1 % triethylamine) to give an oily mixture of products **15a** & **15b** 0.72g (mass recovery 34 %).

**15a** $^1$H NMR DMSO (δ) 2.78 (s, 2 H); 3.08 (s, 3 H); 3.44 (m, 2 H); 3.49 (m, 2H),

3.89 (m, 1H); 4.5 (s, 1 H); 7.5 (s, 5 H).

**15b** $^1$H NMR DMSO (δ) 2.78 (s, 2 H); 3.08 (s, 3 H); 3.35 (m, 1 H); 4.10 (m, 4H); 4.5 (s, 1 H); 7.5 (s, 5 H).

***Orthoester monomer diol 21a derived from lactone 21***

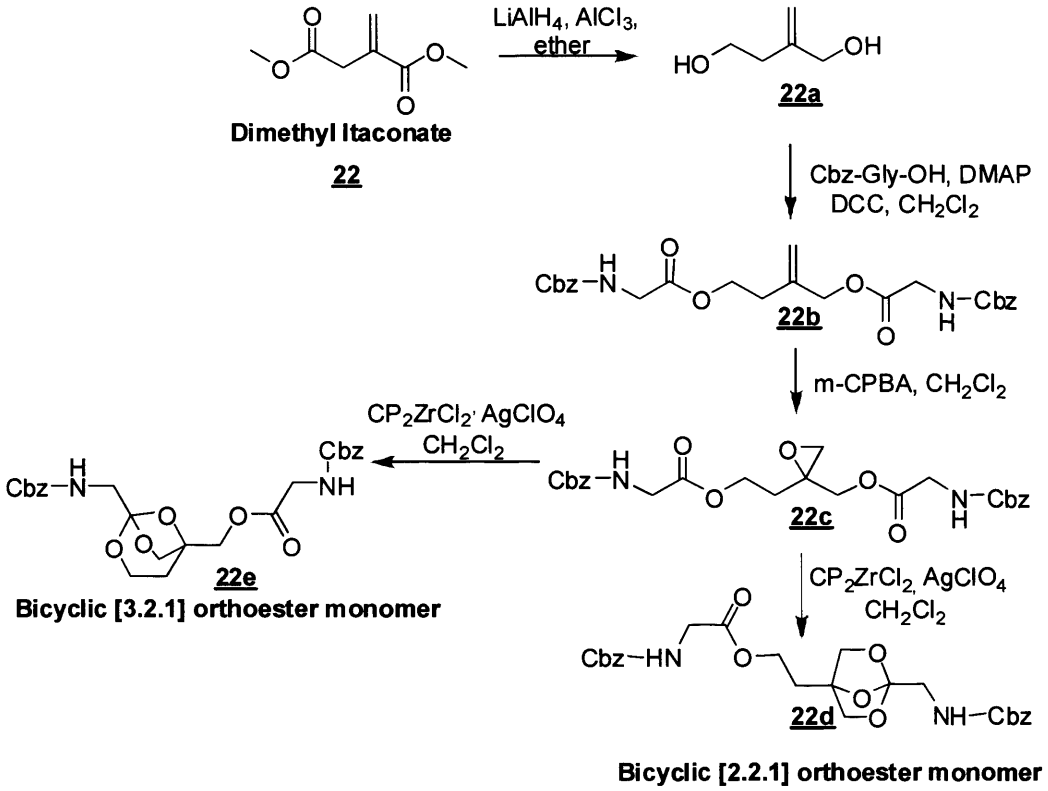



**Scheme 11:** Orthoester monomer **21a** derived from a lactone **21** and a triol.

A stirred solution of lactone **21** (1.5 g, 9.60 mmol) in toluene (40 ml) was fitted with a Dean-Stark apparatus and a $CaCl_2$ guard. To the solution freshly distilled glycerol (0.77 ml, 10.57 mmol) was added, followed by catalytic amount of camphor sulphonic acid (0.02 g, 0.09 mmol). The reaction was refluxed until theoretical amount of water was collected, and then neutralized with triethylamine (0.5 ml). The solution was filtered through celite and concentrated in a vacuo. The crude product was redissolved in ethyl acetate (100ml), washed with saturated sodium bicarbonate (2 x 30 ml), dried over $Na_2SO_4$ and concentrated in vacuo. The residue was chromatographed on silica (pre-treated with 1 % triethylamine) ((chloroform / methanol (9:1)) with 1 % triethylamine) to give a clear oil as **21a** 0.350g (mass recovery 12 %).

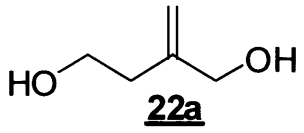
**21a** $^1$H NMR DMSO (δ) 1.34 (dd, 4 H); 2.09 (dd, 4 H); 3.44 (m, 4 H); 3.90 – 4.10 (m, 6H); 4.67 (s, 2 H).

*itaconic acid derived orthoester monomer synthesis*




**Scheme 12:** Synthesis of itaconic acid derived orthoester monomer molecule **22d** and **22e**.

*2-methylene butane-1,4-diol* **22a**




A solution of dimethyl itaconate **22** (1.5g ml, 9. 48 mmol) in ether (10 ml) was left to stir at 0 °C for 10 min[131]. In a separate flask, anhydrous $AlCl_3$ 2.53 g (18.97 mmol) was suspended in ether (5 ml). To the suspension a solution of $LiAlH_4$ (56.96 ml (1M con$^{c.}$)) was added drop-wise at 0 °C over 15 min. To this solution the dimethyl itaconate **22** solution was added drop-wise at 0 °C over a further 40 min. A solution of $NHCl_4$ (sat.) was added to quench and end the reaction. The crude

mixture was filtered, washed with excess ether and concentrated in a vacuo to give the vinyl diol **22a** 0.15 g (15 %) as a clear oil.

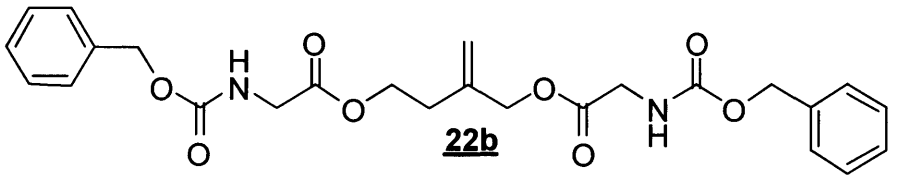
$^1$H-NMR (400 MHz; $(CD_3)_2CO$): δ (ppm) 2.39 (d, 2H), 3.3 (s, 1H), 3.65 (d, 2H), 4.10 (s, 2H), 5.05 (s, 1H), 5.11 (s, 1H).

$^{13}$C-NMR (100 MHz; $(CD_3)_2CO$)): δ (ppm) 39, 61, 66, 115, 147

IR (neat, $cm^{-1}$): 3310 (br), 2960 (s), 2882 (w), 1643 (m), 1385 (m), 1038 (m), 906 (s).

Anal. Calcd for $C_5H_{10}O_2$: C, 58.80; H, 9.87; O, 31.33. Found C, 59.20; H, 9.47; O, 32.13.

***2-methylenebutane-1,4-diyl bis (2-(benzyloxycarbonylamino) acetate)*** **22b**



**22b**

A solution of CBz-Gly-OH (0.62g ml, 2.94 mmol) in $CH_2Cl_2$/DMF (9:1) was added drop-wise to a cold solution of DCC (0.60 g, 2.94 mmol) in $CH_2Cl_2$ (10 ml). The reaction was brought to room temperature slowly for 1 hr and left to stir for further 2 hrs. The solution was filtered, washed with 1 % $NH_4Cl$ (2 x 50 ml), 5 % $NaHCO_3$ (50 ml), water (2 x 50 ml), NaCl solution (sat.), dried over $Na_2SO_4$ and concentrated in a vacuo to give 0.67g as crude mixture. The residue was purified via chromatography on $SiO_2$ (ethyl acetate/hexane, 2:1) to give 0.33 g (47 %) of **22b** as white foam.

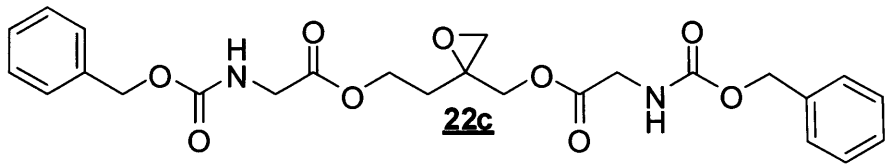
$^1$H-NMR (400 MHz; $(CDCl_3)$): δ (ppm) 2.38 (d, 2H), 3.87 (d, 2H), 3.90 (s, 2H), 3.96 (m, 2H), 4.80 (s, 2H), 5.10 (s, 1H), 5.12 (s, 1H), 7.34 (m, 5H), 8.06 (s, 1H).

$^{13}$C-NMR (100 MHz; $(CDCl_3)$): δ (ppm) 39.6, 40.6, 65.5, 66.1, 69.3, 127.9 - 128.5, 140.1, 148.9, 160.5, 174.9.

IR (neat, $cm^{-1}$): 3354 (sharp, w), 2971 (w), 2802 (w), 1761 (s), 1692 (s), 1643 (m), 1607 (m), 1450 (m), 1206 (w), 906 (m).

Anal. Calcd for $C_{25}H_{28}N_2O_8$: C, 61.97; H, 5.83; N, 5.78, O, 26.42. Found C, 61.67; H, 5.43; N, 5.38; O, 25.82.

***(2-(2-(2-(benzyloxycarbonylamino)acetoxy)ethyl)oxiran-2-yl)methyl2-(benzyloxycarbonylamino)acetate*** **22c**



**22c**

A stirred solution of glycine diester **22b** (0.33g, 0.69 mmol) in THF and $CH_2Cl_2$ (10 ml) was added portion wise *m*-chloroperoxybenzoic acid (0.58 g, 3.38 mmol) and left to stir overnight. The reaction was filtered through celite and concentrated, then diluted with ethyl acetate (30 ml), washed with 15 % NaOH (aq. 10 ml x 2), brine (10 ml) and dried over $Na_2SO_4$, and concentrated in a vacuo. The residue was purified via chromatography on $SiO_2$ (ethyl acetate/hexane, 2:1) to give 0.15 g (42 %) of **22c** as a white solid.

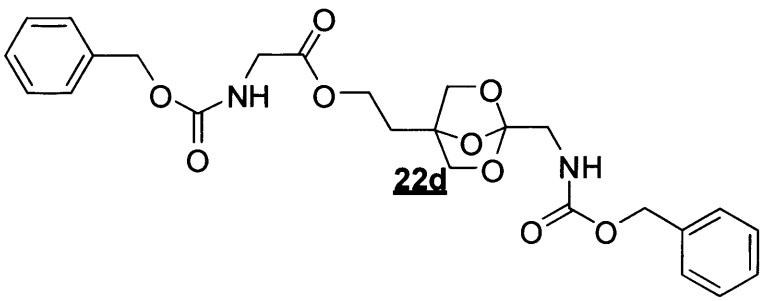
$^1$H-NMR (400 MHz; ($CDCl_3$): δ (ppm) 2.16 (d, 2H), 2.59 (s, 2H (epoxide)), 3.65 (d, 2H), 3.96 (s, 2H), 4.10 (m, 2H), 4.77 (s, 2H), 7.24 (m, 5H), 8.10 (s, 1H).

$^{13}$C-NMR (100 MHz; ($CDCl_3$): δ (ppm) 38.1, 40.4, 49.0, 55.09 (epoxide), 66.4, 66.1, 69.3, 126.8-129.09, 139, 163.1, 172.8.

IR (neat, $cm^{-1}$): 3329 (sharp, w), 2966 (w), 2878 (w), 1759 (s), 1676 (s), 1634 (m), 1600 (m), 1403 (m), 1234 (w), 910 (m).

Anal. Calcd for $C_{25}H_{28}N_2O_9$: C, 59.99; H, 5.64; N, 5.60, O, 28.77. Found C, 60.39; H, 5.94; N, 6.20 ; O, 29.57.

***2-(1-((benzyloxycarbonylamino)methyl)-2,6,7-trioxabicyclo[2.2.1]heptan-4-yl)ethyl 2-(benzyloxycarbonylamino) acetate*** **22d**



**22d**

A solution of epoxy diester **22c** (0.13 g, 0.26 mmol) in $CH_2Cl_2$ (2 ml) was purged with a flow of argon. To the solution $CP_2ZrCl_2$ (8 mg, 0.03 mmol) and $AgClO_4$ (1 mg, 0.0052 mmol) was added. The reaction mixture was stirred at room temperature for 4 hrs under a constant flow of argon. The reaction was stopped by pouring the mixture into aqueous $NaHCO_3$ solution (sat.) and extracted with ethyl acetate (3 x 10 ml). The combined organic layers was dried over $Na_2SO_4$, filtered and concentrated in a vacuo. The residue was purified via chromatography on $SiO_2$, which was pre-treated with 1 % triethylamine (acetone/hexane, 2:1 (1 % triethylamine in mobile phase)) to give 20 mg (12 %) of **22d** as white solid.

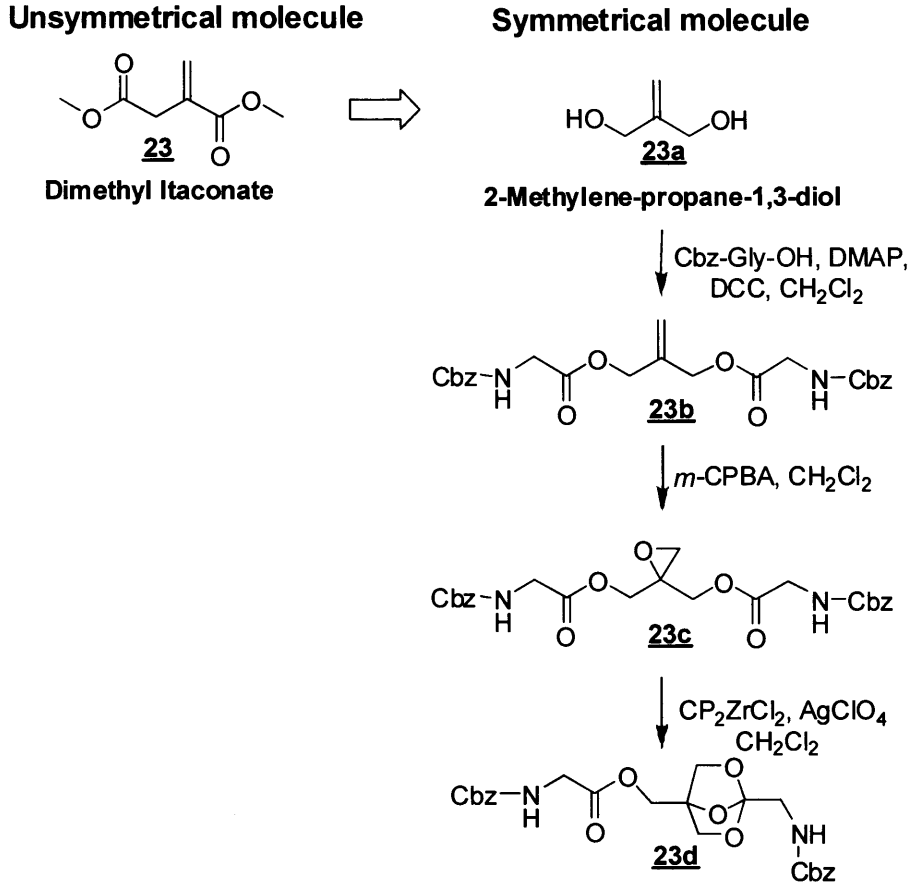
$^1$H-NMR (400 MHz; ($CDCl_3$): δ (ppm) 1.72 (d, 2H), 3.56 (d, 2H), 3.86 (d, 2H), 3.92 (s, 2H), 4.10 (s, 2H (orthoester)), 4.64 (s, 2H), 6.72 (s, 1H), 7.34 (m, 5H), 8.02 (s, 1H).

$^{13}$C-NMR (100 MHz; ($CDCl_3$): δ (ppm) 30.0, 39.2, 55.0 (orthoester), 66.5, 69.3, 70.33 (orthoester), 82.3 (**C**-orthoester) 123.5 (orthoester-**C**-$CH_2NH$), 124.6-128, 138, 162.7, 163.0, 175.2 6.

IR (neat, $cm^{-1}$): 3307 (sharp, w), 2958 (w), 2812 (w), 1731 (s), 1656 (s), 1612 (m), 1334 (m), 1123 (w), 1089(w), 966 (m).

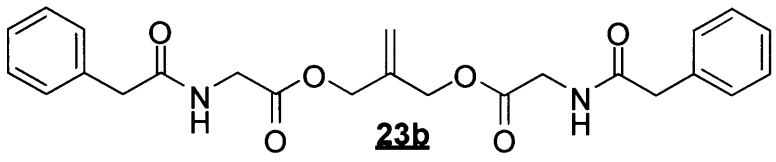
Anal. Calcd for $C_{25}H_{28}N_2O_9$: C, 59.99; H, 5.64; N, 5.60, O, 28.77. Found C, 59.79; H, 5.24; N, 5.10; O, 28.02.

*2-Methylene-propane-1,3-diol derived orthoester monomer molecule synthesis*




**Scheme 13:** Synthesis of 2-Methylene-1, 3-propane diol **23a** derived orthoester monomer molecule **23d**[92].

*2-methylenepropane-1,3-diyl-bis(2-(2-phenylacetamido)acetate* **23b**




A solution of Cbz-Gly-OH (2.37g, 11.35 mmol), dimethylaminopyridine (0.069 g, 0.57 mmol), and 2-methylene propanediol **23a** (0.5g, 5.67 mmol) in $CH_2Cl_2$ / DMF (9:1) (5 ml) was added drop-wise to a cold solution of DCC 3.04 g (14.75 mmol) in $CH_2Cl_2$ (2 ml). The reaction was slowly brought to room temperature for 1 hr and

left to stir for a further 2 hrs. The solution was filtered, washed with 1 % $NH_4Cl$ (2 x 50 ml), 5 % $NaHCO_3$ (50 ml), water (2 x 50 ml), NaCl solution (sat.), dried over $Na_2SO_4$ and concentrated in a vacuo. The residue was purified via chromatography on $SiO_2$ (ethyl acetate/hexane, 2:1) to give 2.16 g (87 %) of **23b** as white foam.

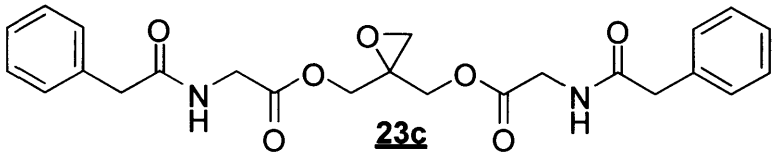
$^1$H-NMR (400 MHz; ($CDCl_3$): δ (ppm) 3.90 (s, 2H), 3.96 (m, 2H), 4.80 (s, 2H), 5.08 (s, 1H), 5.02 (s, 1H), 7.10 (m, 5H), 8.02 (s, 1H).

$^{13}$C-NMR (100MHz;($CDCl_3$):δ(ppm) 39.2, 65.5, 68.1, 109.4, (127.0 -128.1, 130.8, 147.8, 162.3, 175.2.

IR (neat, $cm^{-1}$): 3334 (sharp, w), 2934 (w), 2804 (w), 1760 (s), 1680 (s), 1643 (m), 1601 (m), 1210 (w), 934 (m).

Anal. Calcd for $C_{24}H_{26}N_2O_6$: C, 65.74; H, 5.98; N, 6.39, O, 21.89. Found C, 65.34; H, 5.68; N, 5.89; O, 20.99.

***Oxirane-2,2-diyl bis(methylene) bis(2-(2-phenylacetamido)acetate* 23c**



**23c**

A stirred solution of glycine derived diester **23b** (0.23 g, 0.36 mmol) in $CH_2Cl_2$ and THF (2 x 2 ml) was added to a solution of *m*-chloroperoxybenzoic acid (0.31 g, 1.78 mmol) in $CH_2Cl_2$ (2 ml) slowly and left to stir overnight. The reaction was concentrated, then diluted with 30 ml ethyl acetate, washed with 15 % NaOH (aq. 5 ml x 2), brine (5 ml) and dried ($Na_2SO_4$), and concentrated in a vacuo. The residue was purified via chromatography on $SiO_2$ (ethyl acetate/hexane, 2:1) to give 0.18 g (79 %) of **23c** as a white solid.

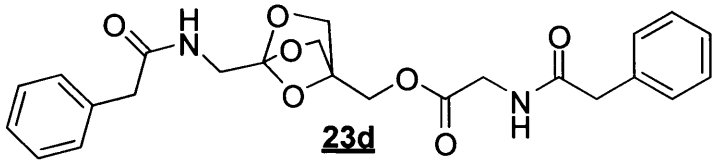
$^1$H-NMR (400 MHz; ($CDCl_3$): δ (ppm) 2.54 (s, 2H), 3.90 (s, 2H), 3.92 (m, 2H), 4.78 (s, 2H), 7.34 (m, 5H), 8.13 (s, 1H).

$^{13}$C-NMR (100MHz ;( $CDCl_3$): δ (ppm) 38.0 (epoxide), 40.4, 64.5 (epoxide), 66.2, 110.12 (epoxide), 127.9 -129.0, 132.2, 164.3, 176.2.

IR (neat, $cm^{-1}$): 3367 (sharp, w), 2934 (w), 2821 (w), 1764 (s), 1660 (s), 1646 (m), 1612 (m), 1198 (w), 960 (m).

Anal. Calcd for $C_{24}H_{26}N_2O_7$: C, 63.43; H, 5.77; N, 6.16, O, 24.64. Found C, 64.03; H, 6.27; N, 6.66; O, 25.44.

***(1-((2-phenylacetamido)methyl)-2,6,7-trioxabicyclo[2.2.1]heptan-4-yl)methyl 2-(2-phenylacetamido)acetate* 23d**

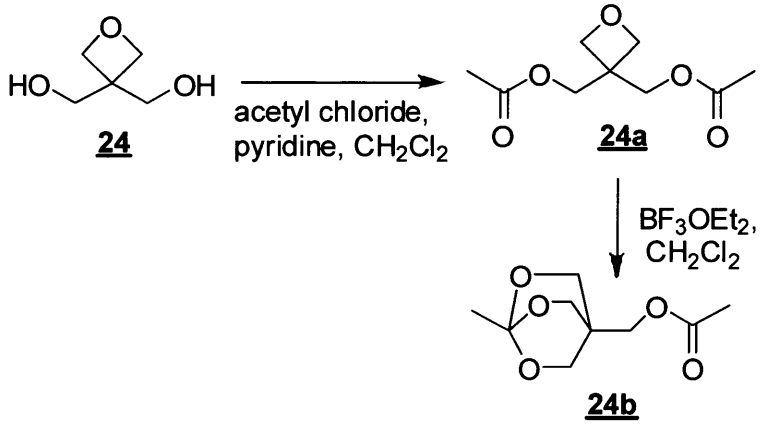


**23d**

To a stirred solution of epoxy diester **23c** (0.100 g, 0.22 mmol) in $CH_2Cl_2$ (2 ml) was added a solution of $CP_2ZrCl_2$ (0.0064 g, 0.02 mmol) and $AgClO_4$ (1 mg, 0.0044 mmol) under a flow of argon. The reaction mixture was stirred at room temperature for 4 hrs, then stopped by pouring the reaction mixture into aqueous $NaHCO_3$ solution (sat.) and extracted with ethyl acetate (3 x 10 ml). The combined organic layers were dried over $Na_2SO_4$, filtered and concentrated in a vacuo. The residue was purified via chromatography on $SiO_2$ that was pre-treated with 1 % triethylamine (acetone/hexane, 2:1 (1 % triethylamine in mobile phase)) to give 12 mg (12 %) of **23d** as white solid:

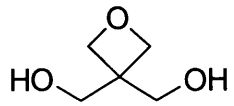
$^1$H-NMR (400 MHz; ($CDCl_3$): δ (ppm)) 3.54 (s, 2H), 3.92 (s, 2H), 4.04 (s, 2H (orthoester)), 4.17 (s, 2H), 6.66 (s, 1H), 7.58 (m, 5H), 8.21 (s, 1H). **23d** was unstable to analyse further.

***Model bicyclic [2.2.2] orthoester derived from acetyl chloride***




**Scheme 14:** Synthesis of model bicyclic [2.2.2] orthoester **24b** derived from oxetane diol **24**.

*(3-Hydroxymethyl-oxetan-3-yl)-methanol)* **24**

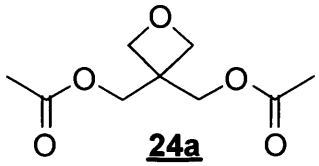


**24**

A solution of potassium hydroxide (7.34 g, 130.9 mmol) in absolute ethanol (80 ml) was added to a solution of pentaerythritol monobromide (20.0 g, 100.6 mmol) in absolute ethanol (80 ml). The mixture was stirred at room temperature for 2 hrs, refluxed on a steam bath for 5 min, then cooled in an ice bath and filtered to remove potassium bromide. The solution was neutralised with acetic acid (1 M) and concentrated in vacuo to give 12.4g (91 %) of **24** as a clear oil. The crude material was then distilled under vacuum (0.5 Hg/mm) at 180 °C to give **24** as a semi solid material.

$^1$H-NMR (400 MHz; $(CD_3)_2CO$): δ (ppm) 3.62 (s, 2*H*, C*H*$_2$OH), 4.10 (s, 1H, $CH_2OH$), 4.39 (s, 2*H*, oxetane). No further analysis was under taken, as oxetane diol **24** was widely analysed within literature.

*Acetic acid 3-acetoxymethyl-oxetan-3-ylmethyl ester* **24a**



**24a**

A stirred solution of oxetane diol **24** (0.5 g, 4.23 mmol), pyridine (0.76 ml, 9.36 mmol) in $CH_2Cl_2$ (3 ml), was added a solution of acetyl chloride (0.66 ml, 9.31 mmol) in $CH_2Cl_2$ (2 ml) slowly at 0 °C. The reaction mixture was allowed to warm to room temperature overnight while stirring. The reaction was diluted with $CH_2Cl_2$ (20 ml), then washed with water (10 ml x 2), brine (10 ml x 2), dried over ($Na_2SO_4$), and filtered. The solution then was concentrated in a vacuo to give 0.54g as crude mixture. The residue was purified via chromatography on $SiO_2$ that was pre-treated

with 1 % triethylamine (ether/hexane 1:1) and to give 0.32 g (38 %) of **24a** as a colourless oil: $R_f = 0.24$

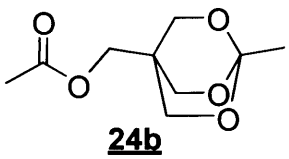
$^1$H-NMR (400 MHz; $(CD_3)_2CO$): δ (ppm) 2.05 (s, 3H, $CH_3CO$), 4.31 (s, 2H), 4.49 (s, 2H).

$^{13}$C-NMR (100 MHz; $(CD_3)_2CO$)): δ (ppm) 171.60, 75.99 (oxetane), 66.02, 43.53, 21.17.

IR (neat, $cm^{-1}$): 2960 (w), 2882 (w), 1739 (s), 1366 (m), 1225 (w), 1038 (m), 986 (m).

Anal. Calcd for $C_9H_{14}O_5$: C, 53.46; H, 6.98; O, 39.56. Found C, 53.68; H, 7.28; O, 39.27.

***Acetic acid 1-methyl-2,6,7-trioxa-bicyclo[2.2.2]oct-4-ylmethyl ester* 24b**



**24b**

To a stirred solution of oxetane diester **24a** (0.10 g, 0.50 mmol) in $CH_2Cl_2$ (2 ml), under a flow of argon was cooled to – 40 °C (ethylene glycol / $CO_2$) and a diluted solution of $BF_3OEt_2$ in $CH_2Cl_2$ (0.05 con$^c$) (387.5 μL, 0.15 mmol) was injected drop wise. The reaction was stirred for 72 h at -18 °C and followed by TLC. Once the reaction complete it was purified via chromatography on $SiO_2$ (ether/hexane, 4:1) with 1 % triethylamine in the mobile phase. This produced 40 mg (40 %) of **24b** as a colourless semi-solid.

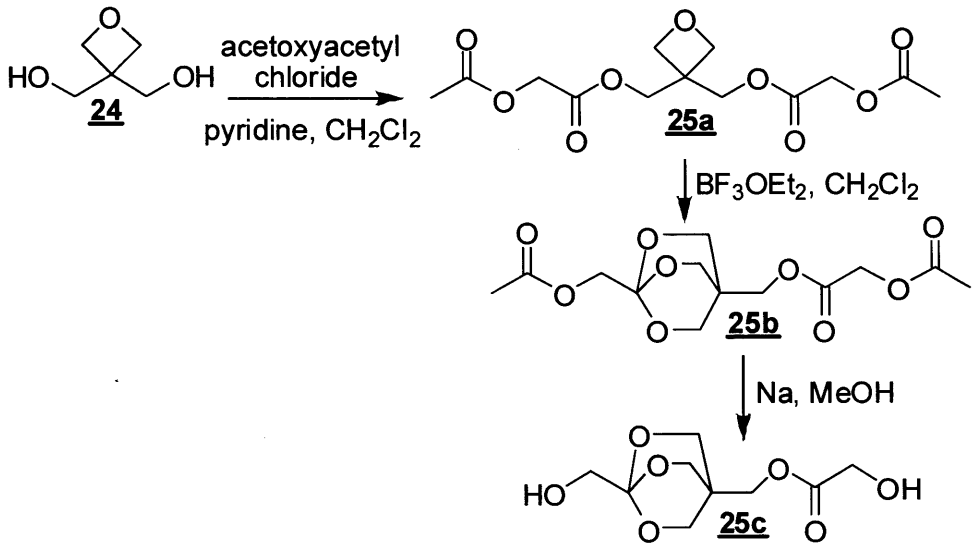
$^1$H-NMR (400 MHz; $(CD_3)_2CO$): δ (ppm) 4.01 (s, 2H), 3.96 (s, 2H), 2.07 (s, 3H), 1.34 (s, 3H).

$^{13}$C-NMR (100 MHz; $(CD_3)_2CO$)): δ (ppm) 171.13, 110.3O, 70.33, 63.31, 35.80, 24.71, , 21.31.

IR (neat, $cm^{-1}$): 2968, 2862, 1738, 1361, 1125, 1041, 966.

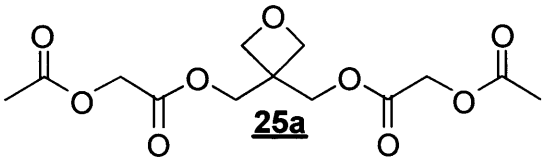
Anal. Calcd for $C_9H_{14}O_5$: C, 53.46; H, 6.98; O, 39.56; Found C, 53.63; H, 7.36; O, 39.71.

*Glycolic acid derived bicyclic [2.2.2] orthoester monomer molecule synthesis*




**Scheme 15:** Glycolic acid derived bicyclic [2.2.2] orthoester monomer **25c** synthesis.

*Oxetane-3,3-diylbis(methylene)bis(2-acetoxyacetate)* **25a**




To a stirred solution of oxetane diol **24** (1.0 g, 8.47 mmol), pyridine (1.5 ml, 18.63 mmol) in THF (10 ml) at 0 °C was injected with a solution of acetyl chloride (2 ml, 18.63 mmol) drop-wise. The reaction was allowed to warm to room temperature slowly and followed by TLC. Once the reaction was complete it was concentrated in a vacuo. The concentrated residue was diluted with ethyl acetate (20 ml) and washed with water (10 ml x 2), brine (10ml x 2), dried over ($Na_2SO_4$), and filtered. The crude product mixture was purified via chromatography on $SiO_2$ (ether/hexane (1:1) and 1 % triethylamine) to give 1.16 g (43 %) of **25a** as a colourless oil:

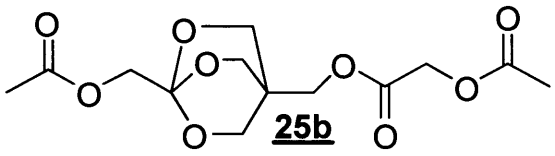
$^1$H-NMR (400 MHz; $(CD_3)_2C=0$): δ (ppm) 2.19 (s, 3H), 4.44 (d, 2H,), 4.48 (d, 2H,), 4.72 (d, 2H,).

$^{13}$C-NMR (100 MHz; $(CD_3)_2CO$)): δ (ppm) 171.60, 169.2, 75.99, 66.15, 60.56, 44.63, 20.19.

IR (neat, $cm^{-1}$): 2965 (w), 2892 (w), 1729 (s), 1356 (m), 1235 (w), 1021 (m), 987 (m).

Anal. Calcd for $C_{13}H_{18}O_9$: C, 49.06; H, 5.70; O, 45.24. Found C, 49.66; H, 6.20; O, 46.14.

***(1-(acetoxymethyl)-2,6,7-trioxabicyclo[2.2.2]octan-4-yl)methyl-2-acetoxyacetate* <u>25b</u>**




To a stirred solution of oxetane diester **<u>25a</u>** (0.205 g, 0.64 mmol) in $CH_2Cl_2$ (4 ml) was cooled down to – 40 °C (ethylene glycol / $CO_2$) and a diluted solution of $BF_3OEt_2$ in $CH_2Cl_2$ (0.05 con$^c$) (500 μL, 0.19 mmol) was injected drop wise. The reaction was purged with a flow of argon, stirred for 72 h at -18 °C and followed by TLC. Once the reaction was complete it was purified via chromatography on $SiO_2$ (ether/hexane (4:1) and 1 % triethylamine) to give 0.15 g (78 %) of **<u>25b</u>** as a white solid. $R_f$ = 0.32.

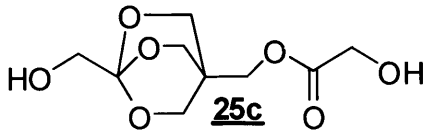
$^1$H-NMR (400 MHz; $(CD_3)_2C$=0): δ (ppm) 2.16 (s, 3H), 3.89 (s, 2H), 4.09 (s, 2H), 4.34 (s, 2H), 4.72 (d, 2H).

$^{13}$C-NMR (100 MHz; $(CD_3)_2CO$)): δ (ppm) 172.40, 169.2, 114.2, 78.12, 70.33, 63.31, 62.92, 37.80, 20.19.

IR (neat, $cm^{-1}$): 2958, 2789, 1731, 1334, 1109, 1092, 966.

Anal. Calcd for $C_{13}H_{18}O_9$: C, 49.06; H, 5.70; O, 45.24. Found C, 48.66; H, 5.30; O, 45.14.

*(1-(acetoxymethyl)-2,6,7-trioxabicyclo[2.2.2]octan-4-yl)methyl-2-acetoxyacetate* **25c**

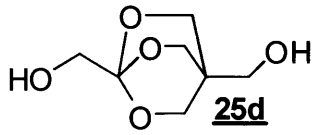



**25c**

To a stirred solution of orthoester diester **25b** (0.80 g, 2.51 mmol) in MeOH (2 ml) was added a solution of sodium (0.11g, 5.02 mmol) in MeOH (2 ml)[132]. The reaction was stirred at room temperature for 16 hrs under a flow of argon, and followed by TLC. Once the reaction was complete half of the reaction mixture was taken out. This was diluted with water (2 ml) and extracted with ethyl acetate (3 ml x 3), dried over $Na_2SO_4$ and concentrated in vacuo. This produced orthoester monomer **25c** as a brown oil [The mass recovery was very low to measure and there was only enough material for an $^1$H-NMR]

$^1$H-NMR (400 MHz; (DMSO): δ (ppm) 3.33 (s, 1H), 3.86 (s, 2H), 4.06 (s, 2H, orthoester), 4.44 (s, 2H), 4.56 (s, 2H).

*2,6,7-trioxabicyclo [2.2.2] octane-1,4-diyldimethanol* **25d**

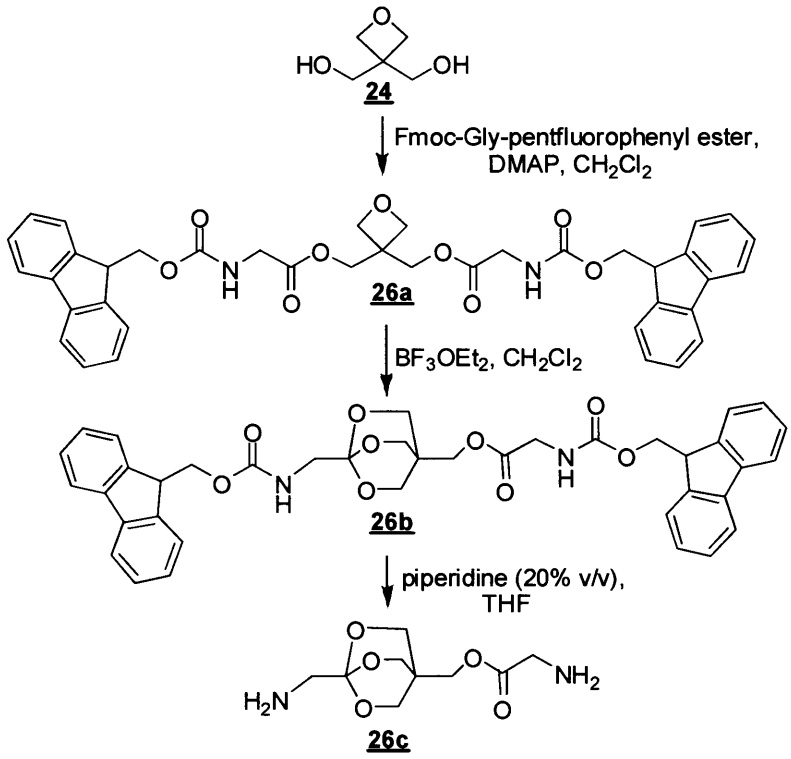



**25d**

The second half of the reaction of orthoester monomer **25c** was continued further for 48hrs. Once the reaction was complete it was diluted with water (2 ml) and extracted with ethylacete (3 ml x 3), dried over $Na_2SO_4$ and concentrated in a vacuo. This produced orthoester monomer **25d** as a colourless oil [as previously with compound **25c** the mass recovery was very low to measure and there was only enough material for an $^1$H-NMR]

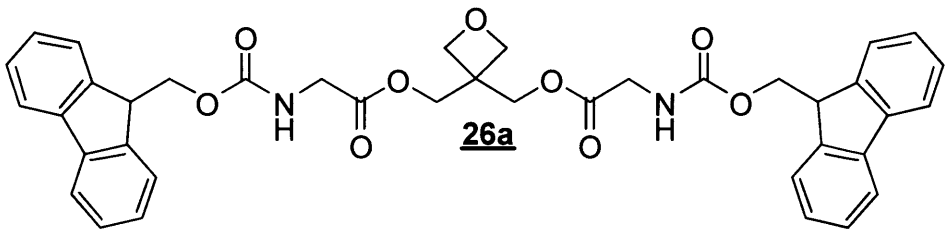
$^1$H-NMR (400 MHz; (DMSO): δ (ppm) 3.35 (s, 1H), 3.78 (s (2H), 3.92 (s, 2H), 4.10 (s, 2H, orthoester).

*L-glycine acid derived bicyclic [2.2.2] orthoester monomer synthesis*




**Scheme 17:** Glycine derived bicyclic [2.2.2] orthoester monomer **26c** synthesis.

***(9H-Fluoren-9-ylmethoxycarbonylamino)-acetic acid 3-[-2-(9H-fluoren-9-ylmethoxycarbonylamino)-acetoxymethyl]-oxetan-3-ylmethyl ester* 26a**




To a stirred solution of oxetane diol **24** (0.41g, 3.48 mmol), dimethylaminopyridine (0.021g, 0.18 mmol), was added in THF (5 ml) and cooled to 0 °C. To this solution a solution of Fmoc-glycine pentafluorophenyl ester (3.23 g, 6.96 mmol) in THF (5 ml) was added drop-wise. The reaction was brought to room temperature slowly and

stirred under a flow of argon overnight. The reaction was followed by TLC. Once the reaction was complete it was concentrated in a vacuo and left under high vacuum (0.05 Hg/mm) to remove excess pentafluorophenol. The crude material was purified via chromatography on $SiO_2$ ($CH_2Cl_2$/acetone (9:1)) to give 1.78 g (76 %) of **26a** as white foam. $R_f = 0.46$.

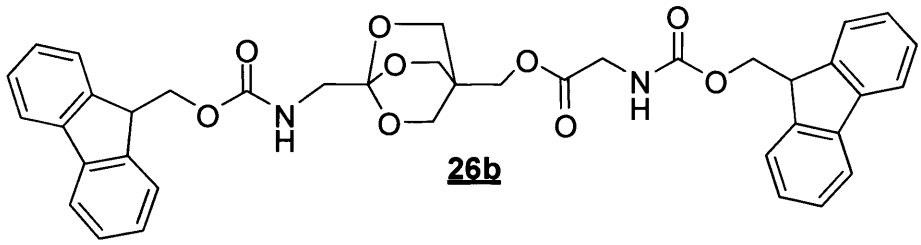
$^1$H-NMR (400 MHz; ($CDCl_3$): δ (ppm) 3.82 (d, 2H), 4.22 (t, 1H), 4.41-4.43 (m, 4H), 4.49 (s, 2H), 5.28 (t, 1H), 7.30 (t, 1H), 7.49 (t, 1H), 7.63 (d, 1H), 7.81 (d, 1H).

$^{13}$C-NMR (100 MHz; ($CD_3)_2CO$)): δ (ppm) 39.65, 47.76, 55.45, 66.10, 66.89, 79.45, 120.04, 125.08, 125.89 ,126.57, 142.93, 143. 07, 154.8, 174.3.

IR (neat, $cm^{-1}$): 3354 (w), 2921 (w), 2851 (w), 1761 (s), 1692 (s), 1540 (m), 1450 (m), 1265 (w), 1175 (m), 649 (m).

Anal. Calcd for $C_{39}H_{36}N_2O_9$: C, 69.22; H, 5.36; N, 4.14; O, 21.28. Found C, 69.44; H, 5.71; N, 3.74; O, 21.41.

***(1-((((9H-Fluren-9-yl) methoxy) carbonylamino) methyl)2,6,7-trioxabicyclo [2.2.2] octan-4-yl) methyl 2-((9-H- fluoren-9-yl) methoxy carbonylamino) acetate*** **26b**



**26b**

To a stirred solution of oxetane diester **26a** (0.200 g, 0.30 mmol) in $CH_2Cl_2$ (3ml) was cooled down to – 40 °C (ethylene glycol / $CO_2$) and a diluted solution of $BF_3OEt_2$ in $CH_2Cl_2$ (0.05 con$^c$) (37.5 μL, 0.014 mmol) was injected drop-wise. The reaction was stirred for 72 h at -18 °C under a flow of argon, and followed by TLC. The crude mixture was filtered, dried over $Na_2SO_4$ and purified via chromatography on $SiO_2$ ($CH_2Cl_2$/acetone (4:1) and 1 % triethylamine) with to give 0.13 g (67 %) of **26b** as white solid foam. $R_f$=0.38.

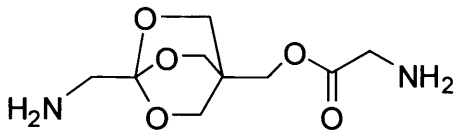
$^1$H-NMR (400 MHz; ($CDCl_3$): δ (ppm) 3.46 (s, 2H), 3. 84 (d, 2H) 3.96 (s, 6H), 4.08 (s, 2H), 4.26 (t, 1H), 4.40 (d, 2H), 5.33 (t, 1H), 7.38 (t, 1H), 7.54 (t, 1H), 7.68 (d, 1H), 7.85 (d, 1H).

$^{13}$C-NMR (100 MHz; $(CD_3)_2CO$)): δ (ppm) 32.58, 47.17, 55.19, 61.51, 65.10, 66.89, 77.21, 108.54, 119.99, 125. 18, 126.99, 127.57, 143.97, 144. 07, 154.1, 176.3.

IR (neat, $cm^{-1}$): 3344 (w), 2931 (w), 2841 (w), 1741 (s), 1682 (s), 1529 (m), 1044 (s), 991 (m), 759 (m).

Anal. Calcd for $C_{39}H_{36}O_9N_2$: C, 69.22; H, 5.36; N, 4.14; O, 21.28. Found C, 69.48; H, 5.49; N, 4.31; O, 21.54.

***(1-(Aminomethyl) -2,6,7-trioxabicyclo [2.2.2] octan-4-yl) methyl 2-aminoacetate*** **26c**

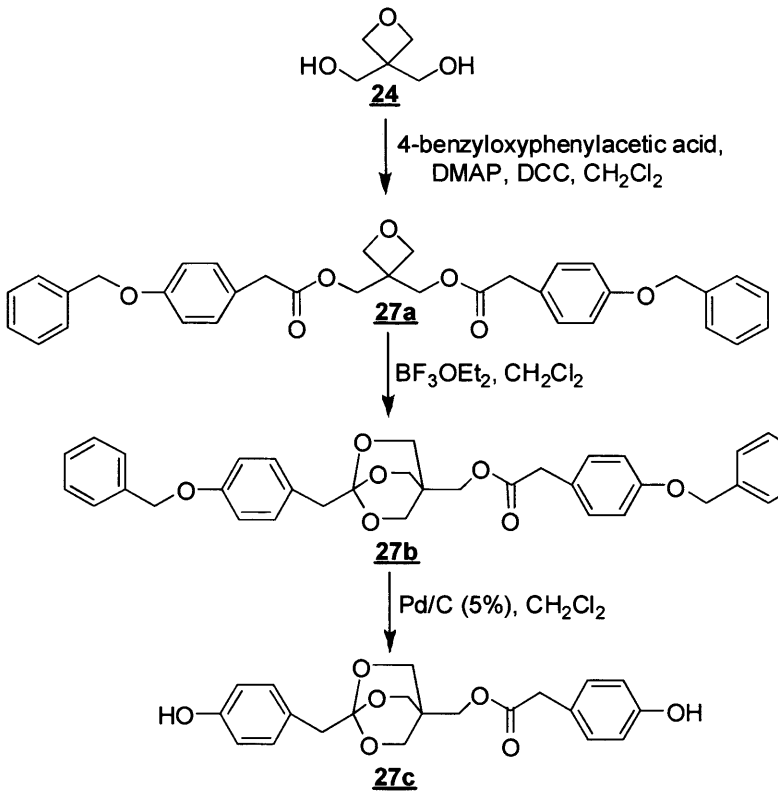



**26c**

To a stirred solution of fmoc orthoester **26b** (0.100 g, 0.15 mmol) in THF (5 ml) was added a solution piperidine 20 % (v/v). The reaction mixtures was stirred at room temperature under a flow of argon until the fmoc group was deprotected (followed by TLC). Once the reaction was complete it was concentrated in a vacuo and purified via chromatography on $SiO_2$ (ethylacete/hexane (4:1) and 1 % triethylamine) to give 6 mg (18 %) of **26c** as oil. $R_f$=0.12

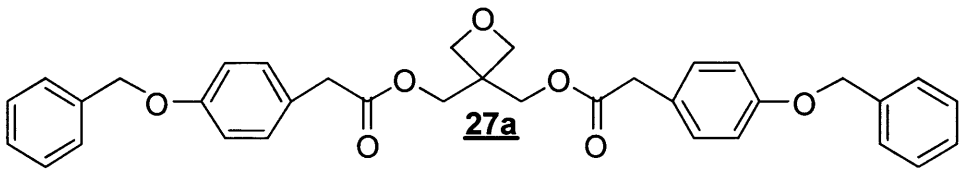
$^1$H-NMR (400 MHz; (DMSO): δ (ppm) 3.46 (s, 2H), 3. 84 (d, 2H), 3.96 (s, 6H), 4.08 (s, 2H), 4.26 (t, 1H), 4.40 (d, 2H), 8.2 (s, 1H). As a result of the low mass recovery it was difficult to do a further analysis e.g. $^{13}$C-NMR of the compound.

***Phenyl acetic acid derived bicyclic [2.2.2] orthoester monomer 27c synthesis***




**Scheme 18:** Phenyl acetic acid derived bicyclic [2.2.2] orthoester monomer 27c synthesis.

***Oxetane -3,3-diylbis (methylene) bis(2-(4-(benzyloxy) phenyl) acetate 27a***




A stirred solution of oxetane diol **24** (1.17g, 9.86 mmol), dimethylaminopyridine (2.41, g19.74 mmol) and 4-benzyloxyphenylacetic acid (4.90 g, 20.23 mmol) in THF (5 ml) and $CH_2Cl_2$ (5 ml) was cooled to 0 °C. To the solution DCC (4.28g, 20.72 mmol) was added and the reaction was brought to room temperature slowly for 1 hr and then left to stir overnight under a flow of argon. The reaction mixture was filtered, washed with 1 % $NH_4Cl$ (2 x 50 ml), 5 % $NaHCO_3$ (50 ml), water (2 x 50 ml), NaCl solution (sat.), dried over $Na_2SO_4$ and concentrated in a vacuo. The

residue was purified via chromatography on $SiO_2$ (ethyl acetate/hexane, 5:5) to give 5.11 g (91 %) of **27a** as a white crystalline material. $R_f$=0.34.

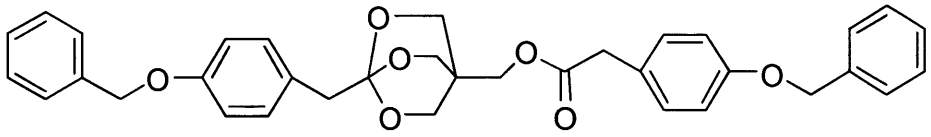
$^1$H-NMR (400 MHz; ($(CH_3)_2S$=O)): δ (ppm) 3.55 (s, 2H), 4.20 (s, 2H), 4.4 (s, 2H), 5.10 (s, 2H), 6.7 (d, 2H), 7.05 (d, 2H), 7.5 (m, 5H).

$^{13}$C-NMR (100 MHz; $(CD_3)_2CO$)): δ (ppm) 38.01, 41.75, 64.57, 72.34, 74.14, 116 – 132.9, 156.19, 171.53.

IR (neat, cm$^{-1}$): 2950 (w), 2822 (w), 1731 (s), 1608 (s), 1265 (m), 1104 (w), 841 (m).

Anal. Calcd for $C_{35}H_{34}O_7$: C, 74.19; H, 6.05; O, 19.76. Found C, 73.69; H, 5.65; O, 18.96.

***(1-(4-(benzyloxy) benzyl)-2,6,7-trioxabicyclo [2.2.2] octan-4-yl) methyl (2-(4-(benzyloxy) phenyl) acetate*** **27b**



**27b**

To a stirred solution of oxetane diester **27a** (0.44 g, 0.77 mmol) in $CH_2Cl_2$ (5 ml) was cooled to .– 40 °C (ethylene glycol / $CO_2$) and a diluted solution of $BF_3OEt_2$ (24 µL, 0.0039 mmol) in $CH_2Cl_2$ (2 ml) (0.05 con$^c$) was injected drop-wise and stirred under a flow of argon. Then reaction was slolwly brought to room temperature, stirred for an additional 2 hrs and followed by TLC until completion. The crude mixture was filtered, dried over $Na_2SO_4$ and purified via chromatography on $SiO_2$ (ethyl acetate/hexane (4:1) and 1 % triethylamine) with to give 0.28 g (65 %) of **27b** as white foam. $R_f$=0. 28.

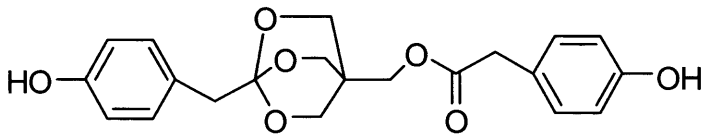
$^1$H-NMR (400 MHz; ($(CH_3)_2S$=O)): δ (ppm) 3.2 (s, 2H), 3.60 (s, 2H), 3.86 (s, 2H), 3.96 (s, 2H), 5.08 (s, 2H), 6.9 (d, 2H), 7.20 (d, 2H), 7.4 (m, 5H).

$^{13}$C-NMR (100 MHz; $(CD_3)_2CO$)): δ (ppm) 36.2, 40.15, 45.60, 62.08, 65.23, 72.98, 110.3O, 114 – 131.2, 138. 34, 154.20, 172.40.

IR (neat, cm$^{-1}$): 2933 (w), 2812 (w), 1786 (s), 1648 (s), 1358, 1212 (m), 1089 (w), 966, 795 (m).

Anal. Calcd for $C_{35}H_{34}O_7$: C, 74.19; H, 6.05; O, 19.76. Found C, 74.69; H, 6. 75; O, 20.41.

***(1-(4-hydroxybenzyl)-2,6,7-trioxabicyclo [2.2.2] octan-4-yl) methyl (2-(4-hydroxyphenyl) acetate*** **27c**




**27c**

To a stirred suspension of orthoester **27b** (0.100 g, 0.17 mmol) in $CH_2Cl_2$ (2 ml) was added palladium carbon (5%) (0.2 g). The sealed reaction system flashed with argon, then kept under hydrogen from a balloon filled with the gas and stirred at room temperature for 73hrs. The reaction was followed by TLC until completion. Once complete the reaction mixture was filtered through celite and the filtrate was washed with THF (2 ml x 2), dried over $Na_2SO_4$ and concentrated in a vacuo. The crude mixture was purified via chromatography on $SiO_2$ (ethyl acetate/hexane (4:1) and 1 % triethylamine) with to give 0.38 g (56 %) of **27c** as white crystalline material. $R_f$= 0.16.

$^1$H-NMR (400 MHz; ($(CH_3)_2S$=O)): δ (ppm) 2.96 (s, 2H), 3.60 (s, 2H), 3.86 (s, 2H), 3.96 (s, 2H orthoester ring), 6.80 (d, 2H), 7.06 (d, 2H), 9.4 (s, 1H).

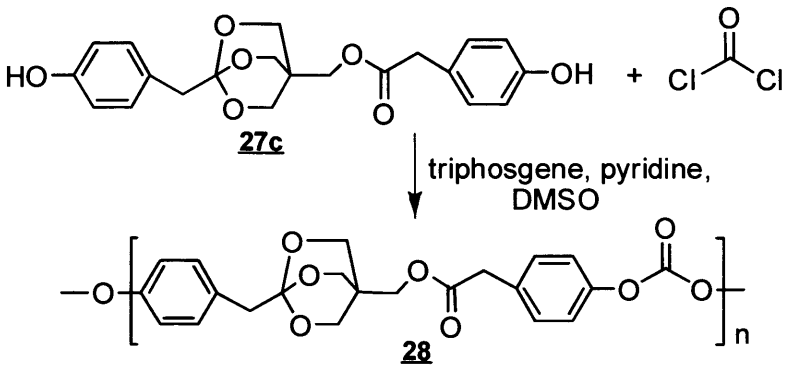
$^{13}$C-NMR (100 MHz; ($(CD_3)_2CO$)): δ (ppm) 33.3, 40.7, 45.12, 62.10, 66.68, 115.82, 127.34, 130.56, 132. 67, 157.0, 171.08.

IR (neat, $cm^{-1}$): 3320 (br), 2912 (w), 2834 (w), 1731 (s), 1600 (s), 1312, 1256 (m), 1065 (w), 934 (w).

Anal. Calcd for $C_{21}H_{22}O_7$: C, 65.28; H, 5.74; O, 28.98. Found C, 64.81; H, 5.18; O, 28.36.

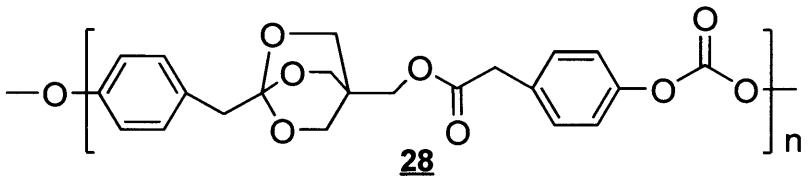
## *Work as described in Chapter 4*

***Phenyl acetic acid derived bicyclic [2.2.2] orthoester monomer used to prepare a new generation of poly(ortho esters)*** **28**




**Scheme 19:** Poly (orthoesters carbonates) **28** synthesis derived from bicyclic [2.2.2] orthoester monomer **27c**.

***Poly (orthoester carbonates)*** **28**




A stirred solution of orthoester monomer **27c** (1.32 g, 3.41 mmol, 1 eq), and pyridine (1.16 ml, 14.36 mmol, 4.2 eq) was added to a oven dried flask in DMSO (3 ml). Which, was then sealed and purged with nitrogen and once the mixture dissolved, the solution was cooled to 0 °C. To the cooled solution a separate solution of triphosgene (3.24 g, 10.93 mmol, 3.2 eq) in toluene (2 ml) was injected drop-wise very slowly. The reaction become vigorous, with a white cloud forming, which became clear as reaction proceeded. The sealed reaction was warmed slowly to room temperature and stirred over 16hrs until the reaction got very viscous. The polymer solution was reduced by concentrating under a flow of argon, then it was diluted in $CH_2Cl_2$ (3 ml) and washed with aqueous ammonium chloride (2 ml x 2). The solution was dried over $Na_2SO_4$, and concentrated. The concentrated solution was

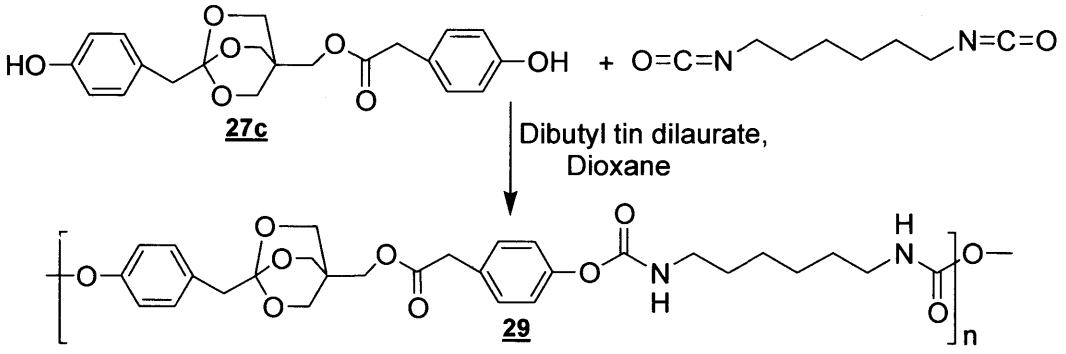
precipitated into a rapidly stirred water (5 ml) to give poly (orthoester carbonates) **28** as a brown oil (MW (7 000 g / $mol^{-1}$) PDI (2.3)). MW measured using conventional GPC calculation against polystyrene standards in THF solvent system.

$^{1}$H-NMR (400 MHz; ($(CH_3)_2S=O$)): δ (ppm) 2.90 (s, 2H), 3.72 (s, 2H), 3.86 (s, 2H), 3.96 (s, 6H orthoester ring), 7.20 – 7. 42 (m, 4H).

$^{13}$C-NMR (100 MHz; ($(CD_3)_2CO$)): δ (ppm) 33.60, 40.7, 45.80, 62.50, 66.42 (orthoester ring), 116.12, 121.40 – 136.50, 148.60 (carbonates), 171.40.

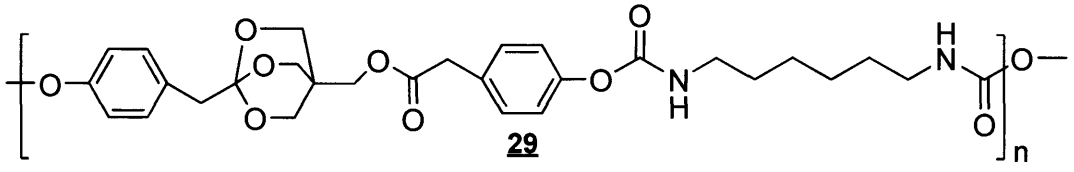
IR (neat, $cm^{-1}$): 2923 (w), 1765 (m) 1731 (m), 1600 (s), 1206, 1042 (w), 914 (w).

***Poly (orthoester urethanes) 29 derived from 4-hydroxy (phenyl acetic) acid bicyclic [2.2.2] orthoesters monomer 27c***




**Scheme 20:** Poly (orthoester urethane) **29** prepared by a polymerisation of an orthoester monomer **27c** and a diisocyante monomer.

***Poly (orthoester urethanes) 29***




A stirred solution of orthoester monomer **27c** (0.2 g, 0.51 mmol, 1 eq), and hexane diisocyanate (91 µL, 0.54 mmol, 1.05 eq) was added to a oven dried pressure tube in dioxane (3 ml). Which was then sealed and purged with argon, once the mixture has

dissolved the catalyst dibutyl diaurate (15 µL, 0.02 mmol, 0.05 eq) was added drop-wise. The reaction was heated at 80 °C for 3 hrs until it got viscous, then left at 70 °C for a further 16 hrs. The viscous reaction mixture was cooled to room temperature, and diluted with dioxane (2 ml). The polymer was isolated by precipitation in rapidly stirring ether (5 ml) to give poly (orthoester urethane) **29** as a brown solid (MW (22 000 g / $mol^{-1}$) PDI (3.1)). MW measured using conventional GPC calculation against polystyrene standards in THF solvent system.

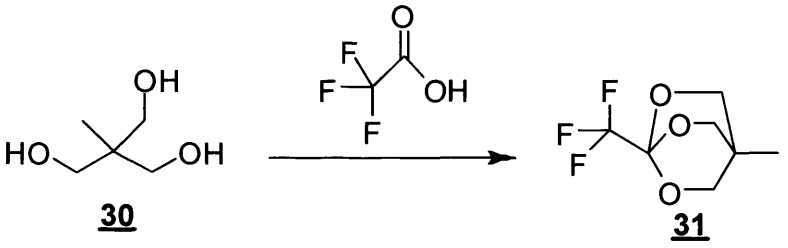
$^{1}$H-NMR (400 MHz; ($(CH_3)_2S=O$)): δ (ppm) 1.28 (m, 4H), 1.46 (m, 4H), 3 06 (s, 2H), 3.20 (m, 4H), 3.68 (m, 2H), 3.92 (m, 2H), 4.10 (m, 6H orthoester ring), 6. 76 (s, 1H), 6.88 – 7.20 (m, 4H).

$^{13}$C-NMR (100 MHz; ($(CD_3)_2CO$)): δ (ppm) 26.89, 32.80, 39.10, 44.60, 61.90, 68.09 (orthoester ring), 116.12 (orthoester ring), 123.90 – 150.40, 154.90 (urethane), 172.20 (ester).

IR (neat, $cm^{-1}$): 3324 (s), 2923 (w), 1756 (m) 1716 (m), 1610 (s), 1196, 1066 (w), 867 (w).

## *Work as described in chapter 5*

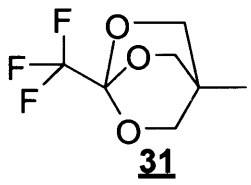
### *Model bicyclic [2.2.2] orthoester derived from 2-hydroxymethyl-2-methyl-1, 3-propanediols*




**30** **31**

**Scheme 21:** Model bicyclic [2.2.2] orthoester **31**.

*4-methyl-1-(trifluoromethyl)-2,6,7-trioxabicyclo[2.2.2] octane* **31**

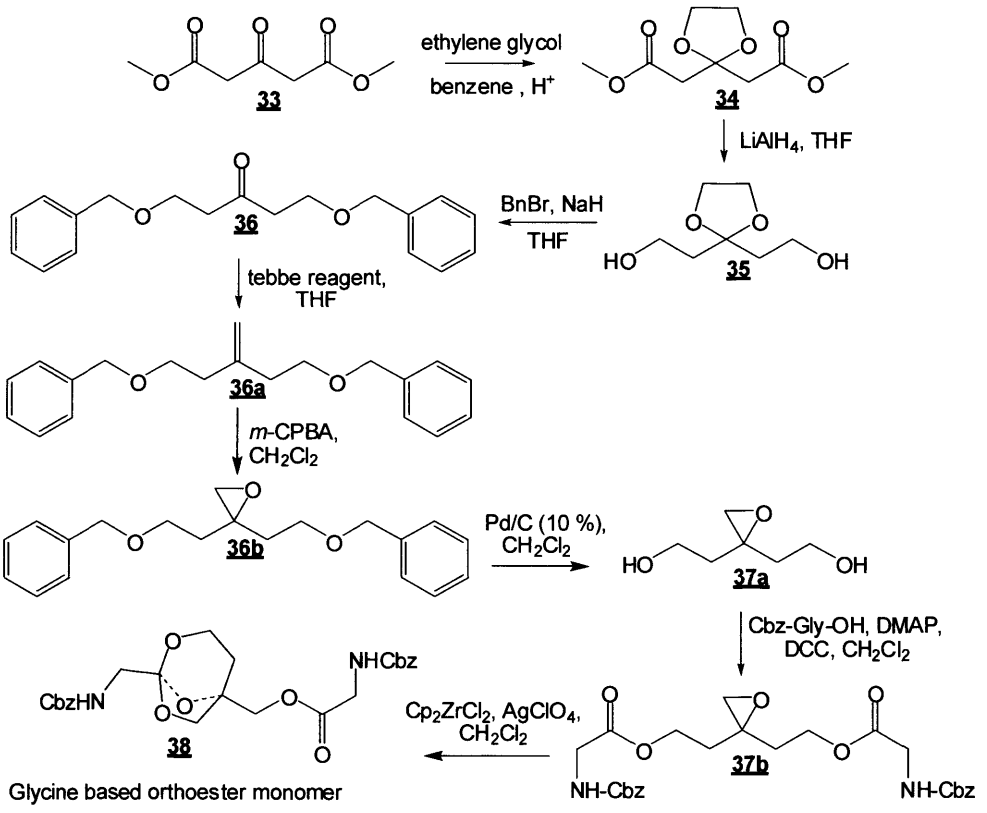


**31**

To a stirred solution of 1,1,1-tris (hydroxymethyl) ethane (6 g, 49 mmol), trichloroacetic acid (8.1 g, 49 mmol) in xylene (40ml) was added the catalyst p-TSA (0.47 g, 5.25 mmol). The reaction was refluxed with azo-tropical removal of the by-product water using a Dean-Stark trap. Once the theoretical amount of water was removed, the reaction was cooled to room temperature, and then further cooled on ice. The cooling subsequent produced a white crystalline precipitate. The crystalline material was washed with sodium carbonate solution to neutralise the acid. The product was isolated by recrystallisation from benzene to give 0.04 g (40 %) of **31** as a white solid:

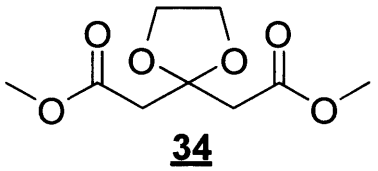
$^1$H-NMR (400 MHz; $(CD_3)_2CO$): δ (ppm) 4.01 (s, 2H), 1.34 (s, 3H).[It should be noted the product degrade rapidly to starting material, if it's not storage under a basic environment] [It should also be noted the compound **31** was not further identified, as the compound was prepared for information gathering only.]

*Bicyclic [3.2.1] orthoester monomer 38 derived from amino acid epoxy ester*




**Scheme 24:** Glycine derived bicyclic [3.2.1] orthoester monomer **38** synthesis based dimethyl acetonedicarboxylate **33**.

*Dimethyl 2, 2-(2, 3-dioxolane-2, 2-diyl)diacetate 34*



**34**

To a stirred solution of dimethyl acetonedicarboxylate **33** (25.2 ml, 127.3 mmol) in benzene (150 ml) was treated with ethylene glycol (16.1 ml, 289.4 mmol) and catalytic amount of boron trifluoride diethyl etherate (0.6 ml, 4.73 mmol). The

reaction mixture was refluxed with azo-tropical removal of the by product water for 24 hrs. The reaction was then washed with aqueous $NaHCO_3$ (10 % w/v) (50 ml), brine (50 ml x2), dried over $Na_2SO_4$ and concentrated in a vacuo. The residue purified via chromatography on $SiO_2$ (ethyl acetate/hexane (1:4) and 2 % triethylamine) with to give 24.4g (65 %) of **34** as white a clear oil. $R_f$=0. 18.

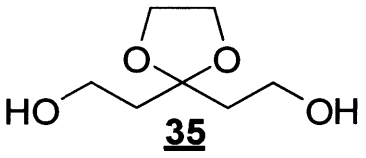
$^1$H-NMR (400 MHz; $CDCl_3$): δ (ppm) 2.96 (s, 2H), 3.70 (s, 3H), 4.02 (s, 2H).

$^{13}$C-NMR (100 MHz; $CDCl_3$): δ (ppm) 41.28, 51.62, 65.09, 106.63, 169.64.

IR (neat, $cm^{-1}$): 2956, 2893, 1736, 1437, 1329, 1033, 640.

MS (El) 218.9 m/z.

***2, 2-(1,3-dioxolane-2,2-diyl) diethanol* 35**




**35**

A stirred solution of the ketal **34** (5.63g, 25.82 mmol) in THF (80 ml) was cooled to 0 °C for 10 min. To the cooled solution a 1M $LiAlH_4$ in THF (77.4 ml, 77.46 mmol) solution was injected drop wise until no hydrogen evolved at 0 °C. Then the reaction was warmed to room temperature slowly, and refluxed for 1 hr. Once the reaction was complete it was cooled to room temperature, and was processed by Fieser's work up, which involved the addition of water (1.4 ml) drop-wise, aqueous NaOH (20.61 ml (15% v/v)) and water (4.2 ml) dropwise at 0 °C. The reaction was further refluxed 10 min, then cooled and extracted with ethyl acetate (50 ml x 3), dried over $Na_2SO_4$ and concentrated in a vacuo. The crude product was purified via

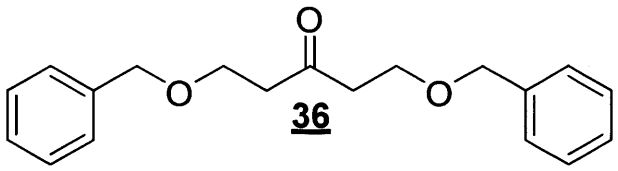
chromatography on $SiO_2$ (ethyl acetate/methanol/ hexane (6:1:3)) to give 3.3 g (79 %) of **35** as a clear oil. $R_f = 0.12$

$^1$H-NMR (400 MHz; $CDCl_3$): δ (ppm) 1.90 (t, 2H), 2.97 (s, 1H), 3.68 (t, 2H) 3.96 (s, 2H).

$^{13}$C-NMR (100 MHz; $CDCl_3$): δ (ppm) 39.20, 58.95, 65.25, 112.21.

IR (neat, $cm^{-1}$): 3355, 2957, 2889, 1139, 1045, 649, 1033, 649.

***1,5-diphenoxypentan-3-one* 36**




**36**

To a stirred solution of ketal diol **35** (0.69g, 4.25 mmol) in THF (3 ml) was added a solution of NaH (0.31g, 12.9 mmol) in THF (2 ml) at 0 °C for 10 min. To the solution benzyl bromide (1.52 ml, 12.9 mmol) in THF (3 ml) of was injected slowly, followed by catalytic amount of KI (5 mg, 0.3 mmol). The reaction was stirred at room temperature for 24 hrs, then concentrated in a vacuo. The concentrated residue was diluted with water (10 ml), extracted with ethyl acetate (10 ml x3), dried over $Na_2SO_4$ and concentrated in a vacuo. The crude benzylated product (1.39g, 4.07 mmol) was dissolved in an co-solvents of THF (10 ml), acetone (7.5 ml) acetone, and aqueous 1M HCl (7.5 ml). The reaction was stirred at 35 °C for 15 hrs, concentrated in a vacuo, then diluted with $CH_2Cl_2$ (50 ml), which was washed with aqueous $NaHCO_3$ ((10 ml x 2) 4% v/v), dried over $Na_2SO_4$ and concentrated in

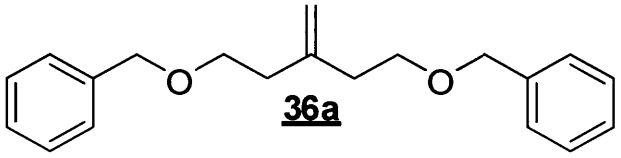
vacuo. The crude product was purified via chromatography on $SiO_2$ (ethyl acetate/hexane, 1:4) to give 1.05 g (83 %) of **36** as a clear oil. $R_f$=0.28.

$^1$H-NMR (400 MHz; $CDCl_3$): δ (ppm) 2.75 (t, 2H), 3.75 (t, 2H), 4.50 (2, 2H) 7.30 (s, 5H).

$^{13}$C-NMR (100 MHz; $CDCl_3$): δ (ppm) 46.10, 66.78, 68.80, 128.90 – 138.12. 210.90.

IR (neat, $cm^{-1}$): 2960, 2802, 1750, 1608, 1108, 1006,

***(3-Methylenepentane1,5-diyl) bis (oxy) dibenzene*** **36a**

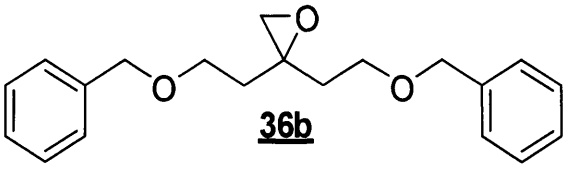


**36a**

To a stirred solution of ketone **36** (0.05g, 0.17 mmol) in THF (2 ml) was added a solution of Tebbe reagent (1.68 ml, 0.84 mmol (0.5 M v/v)) in toluene drop-wise at 0 °C and purged with argon for 1hr. The reaction was then warmed to room temperature slowly for a further 1 hr, then diluted with ether (10 ml). To the diluted solution aqueous NaOH (5-10 drops (0.1M v/v)) and stirred until complete evolution of gas. The solution was dried over $Na_2SO_4$, filtered and concentrated in a vacuo. The crude product was purified via chromatography on $SiO_2$ (ethyl acetate/hexane, 1:4) to give 0.04 g (87 %) of **36a** as a clear oil. $R_f$=0.18

$^1$H-NMR (400 MHz; $CDCl_3$): δ (ppm) 2.37 (t, 2H), 3.58 (t, 2H), 4.50 (2, 2H) 4.85 (s, 1H), 5.10 (s, 1H), 7.33 (m, 5H).

*2,2-bis (2-phenoxyethyl) oxirane* **36b**



**36b**

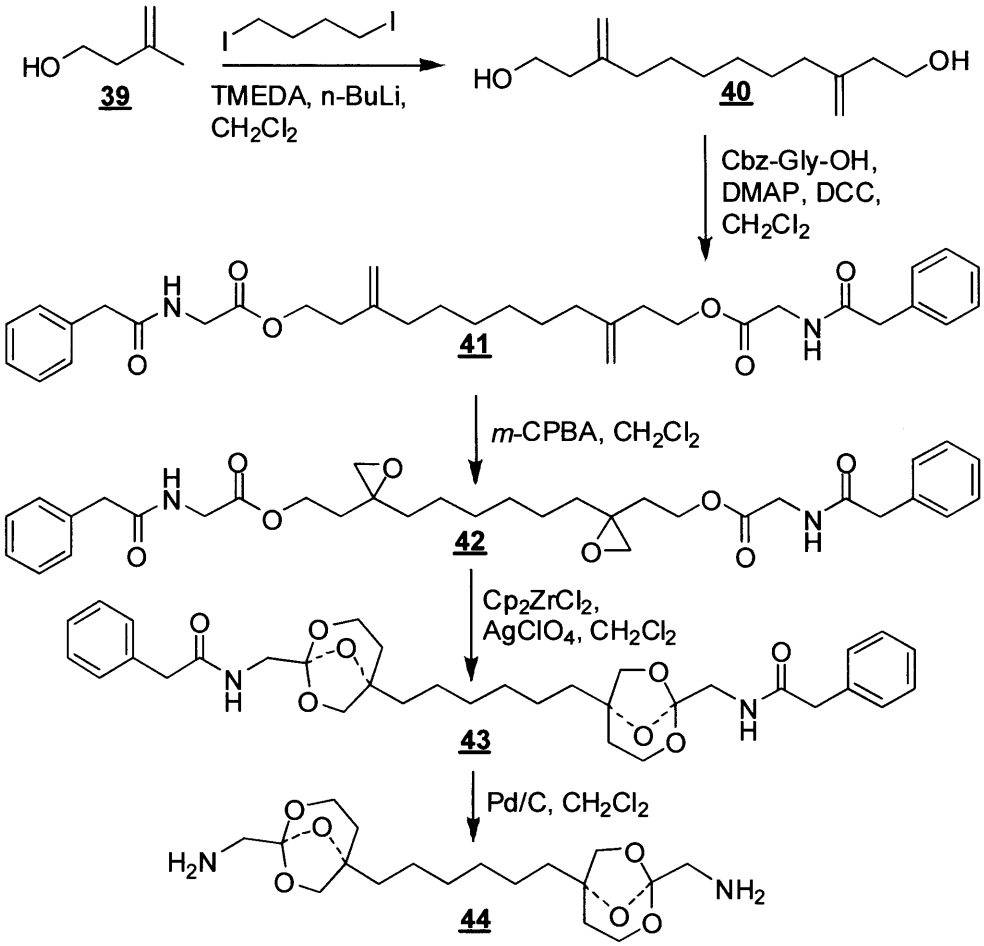
A stirred solution of vinyl diester **36a** (0.22 g, 0.74 mmol) in $CH_2Cl_2$ (2 ml) was added to a solution of *m*-chloroperoxybenzoic acid (0.16 g, 0.89 mmol) in $CH_2Cl_2$ (2 ml) slowly and left to stir overnight. The reaction was concentrated, then diluted with ethyl acetate (10 ml), washed with 15 % NaOH (aq. 3 ml x 2), brine (3 ml) and dried ($Na_2SO_4$), and concentrated in a vacuo. The residue was purified via chromatography on $SiO_2$ (ethyl acetate/hexane, 1:4) to give 0.15 g (66 %) of **36c** as a white solid. $R_f$=0.43.

$^1$H-NMR (400 MHz; $CDCl_3$): δ (ppm) 2.10 (m, 2H), 2. 66 (s, 2H, epoxide), 3.50 (m, 2H), 4.50 (2, 2H), 7.32 (m, 5H).

$^{13}$C-NMR (100 MHz; $CDCl_3$): 33.23, 52. 12, 55. 34, 72. 60, 121.70 – 138.40.

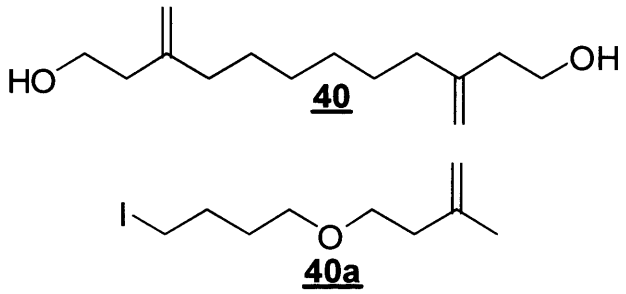
IR (neat, $cm^{-1}$): 2912, 2860, 1608, 1312, 868.

***Di-substituted bicyclic [3.2.1] orthoester derived from 3-methylene butanl-1-ol***




**Scheme 25**: 3-methyl-buten-1-ol **39** derived bicyclic [3.2.1] orthoester **44** monomer synthesis.

***3, 10-Dimethylene-dodecane-1,12-diol* 40**

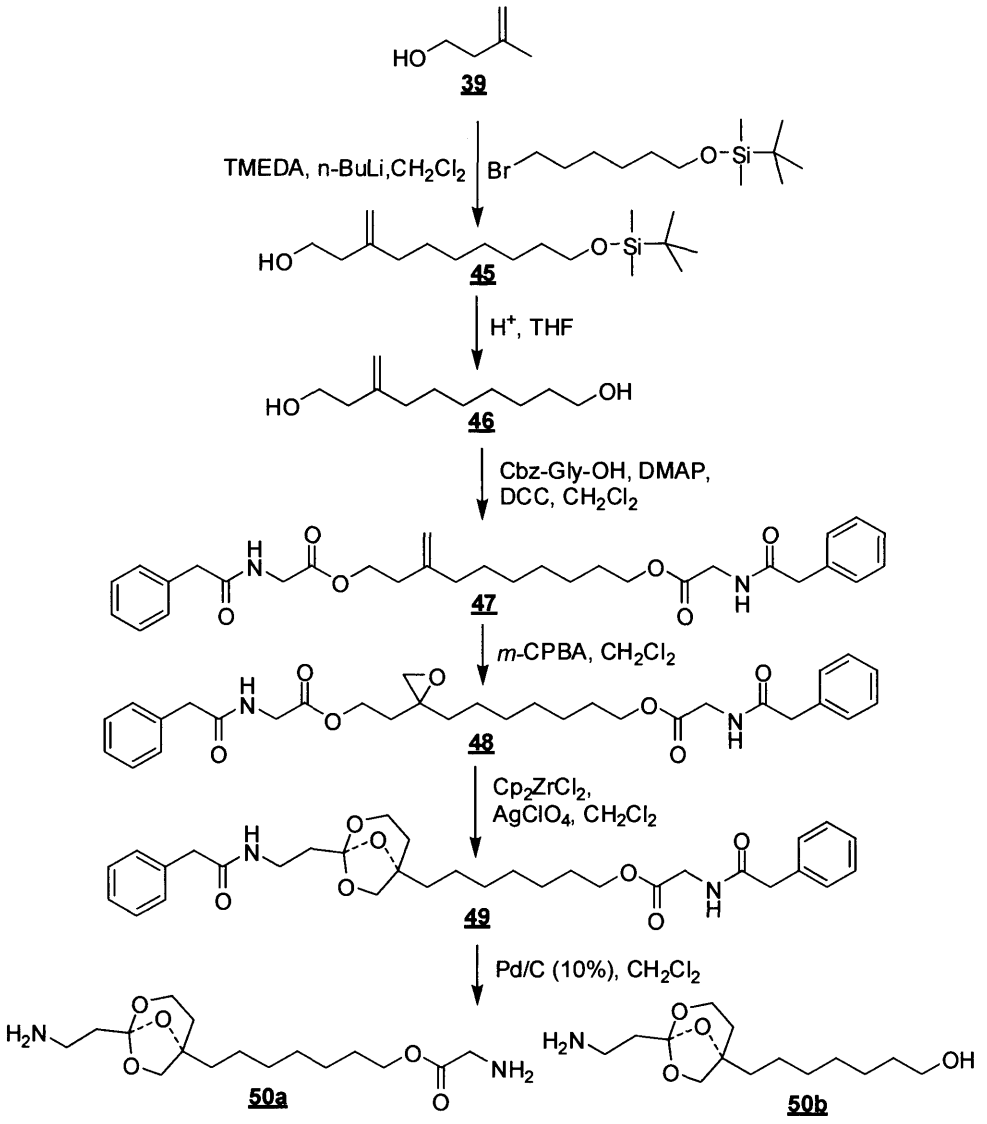



A stirred solution of TMEDA (2.53 ml, 16.77 mmol) in ether (2 ml) was cooled to 0 °C. To the cooled solution n-BuLi (8.10 ml, 12.9 mmol in hexane) was added, and

the resulting solution was stirred at room temperature for 1hr. The solution was then cooled to 0 °C, and alcohol **39** (0.65 ml, 6.45 mmol) was injected slowly. The resulting solution was stirred at room temperature for 6 hrs to generate the desired dianion as a heterogeneous brown suspension. This slurry was cooled to -78 °C and the electrophile 1,4-diiodobutane (0.21 ml, 1.61 mmol) was injected drop-wise. The reaction was warmed to room temperature and was stirred for 16hrs. The reaction was then quenched with aqueous ammonium chlorides (5 ml), and then extracted with ethyl acetate (10 ml x 3). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in a vacuo. The crude product was purified via chromatography on $SiO_2$ (ethyl acetate / hexane (1:4)) to give 0.24 g (56 %) of **40a** as a clear oil. $R_f$= 0.42.

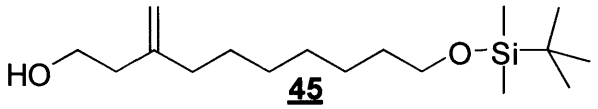
$^1$H-NMR (400 MHz; CDCl3): δ (ppm) 1.67 (m, 2H), 1.94 (m, 4H), 2.24 (m, 2H), 3.20 (m, 2H), 3.50 (m, 4H), 4.92 (s, 1H), and 5.10 (s, 1H).

***Mono-substituted bicyclic [3.2.1] orthoester derived from 3-methylene butan-1-ol***




**Scheme 26:** Mono-substituted and di-substituted bicyclic [3.2.1] orthoester monomer **50a** and **50b**.

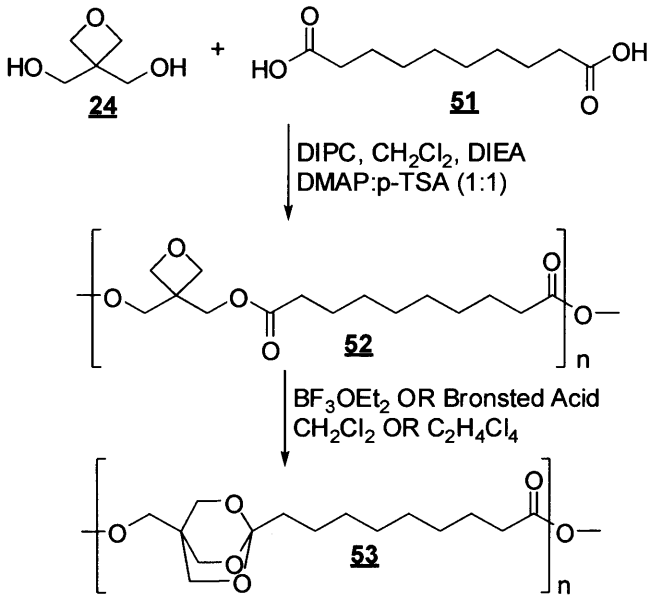
***10-(tert-butyldimethylsilyloxy)-3-methylenedecan-1-ol* 45**




A stirred solution of TMEDA (0.58 ml, 3.89 mmol) in ether (2 ml) was cooled to 0 °C. To the cooled solution n-BuLi (2.12 ml, 3.38 mmol in hexane) was added, and

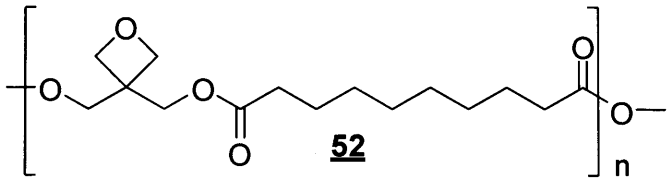
the resulting solution was stirred at room temperature for 1 hr. The solution was then cooled to 0 °C, and alcohol **39** (0.17 ml, 1.69 mmol) was injected slowly. The resulting solution was stirred at room temperature for 6hrs to generate the desired dianion as a heterogeneous brown suspension. This slurry was cooled to -78 °C and the electrophile 6-bromohexyloxy-tert-butyldimethylsilane (0.47 ml, 1.69 mmol) was injected drop-wise. The reaction was warmed to room temperature and was stirred for 16hrs. The reaction was then quenched with aqueous ammonium chlorides (1 ml), and then extracted with ethyl acetate (3 ml x 3). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in a vacuo. The crude product mixture was not purified further, as we were unable to distinguish if the product was formed or not

***pH triggered rearrangement reaction used to prepare poly(ortho esters)* 53**




**Scheme 27:** pH triggered rearrangement reaction of precursor *co*-polymer **52** to prepare a poly(ortho esters) **53**.

*Poly (oxetane esters)* **52**



**52**

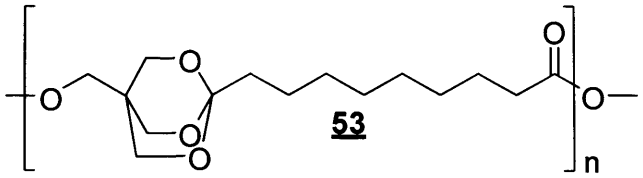
A stirred solution of oxetane diol **24** (2.42 g, 20.50 mmol), and diisopropylethylamine (0.71 ml, 4.10 mmol) was added to a oven dried pressure tube in $CH_2Cl_2$ (20 ml). To the solution 4-(dimethylamino) pyridinium-4-toluenesulfonate (DPTS) (1.21g, 4.10 mmol) and sebacic acid **51** (4.15 g, 20.50 mmol)) was added. The resulting solution was purged with argon, and cooled to 0 ° C and diisopropylcarbodiimide (9.6 ml, 61.51 mmol) was injected dropwise, and the reaction tube was flushed with argon and sealed. The reaction got viscous after 10 min and was stirred under argon for 72 h. The viscous reaction solution was diluted with $CH_2Cl_2$ ( 20 ml) and the polymer was isolated by precipitation in rapidly stirring methanol (100 ml). This produced poly (oxetane esters) **52** as a white solid of 5.33 g (81 % mass recovery). MW 103.08 g $mol^{-1}$ x $10^4$ (PDI 2.36). MW measured using conventional GPC calculation against PMMA standards in THF solvent system.

$^1$H-NMR (400 MHz; $CDCl_3$): δ (ppm) 1.38 (s, 8H), 1.57 (t, 4H), 2.42 (t, 4H), 4.39 (s, 4H), 4.59 (s, 4H).

$^{13}$C-NMR (100 MHz; $CDCl_3$): δ (ppm) 24.84, 29.06, 34.05, 42.15, 64.64, 75.46, 173.61.

IR (neat, $cm^{-1}$) 2930.94 (w), 2857.23(w), 1732.36 (s), 1165.38, 1098.89 (m), 986.62 (oxetane ring) (m).

*Poly (ortho esters)* **53**




**53**

A stirred solution of poly (oxetane ester) **52** (103 mg, 0.45 mmol) in dichloroethane (2 ml) was added to a oven dried pressure tube. To the solution p-TSA (4.2 mg, 0.02 mmol) was added and the reaction was flashed with argon and sealed. The polymer solution heated at 80 °C for 4 hrs, and then stopped via the addition of anhydrous triethylamine (4.5 μL, 0.04 mmol)). The viscous solution reduced under a flow of argon, and purified via precipitation in rapidly stirring methanol (5 ml). The polymer was dried under vacuum to give 61 mg (59 % recovery) of poly (ortho esters) **53.** MW 56.08 g $mol^{-1}$ x $10^4$ (PDI 2.36). MW measured using conventional GPC calculation against PMMA standards in THF solvent system.

$^1$H-NMR (400 MHz; $CDCl_3$): δ (ppm) 1.28 (m, 8H), 1.41 (m, 4H), 2.38 (t, 2H), 2.43 (m, 2H) 3.94 (s, 6H, orthoester ring), 4.02 (s, 2H).

$^{13}$C-NMR (100 MHz; $CDCl_3$): δ (ppm) 21.20, 25.44, 29.26, 38.09, 41.15, 61.03, 68.03, 110.05, 174.78.

IR (neat, $cm^{-1}$) 2932.98 (w), 2810 (w), 1751.20 (s), 1090.10(m), 905.45 (m).

# Chapter 7

## *References*

# Chapter 7

## *References*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